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Clinical Usefulness of Estriol Assay for Predicting "Light-for-Dates" Infants

J. J. Evans, A. R. Wilkinson, and D. R. Aickin

A method was developed for determining clinical usefulness of a test for predicting "light-for-dates" infants before birth. The method was applied in a prospective study of assay of maternal estriol at 35–36 weeks of gestation as a means of identifying such infants. With the tenth centile for estriol values as the cutoff, low estriol values identified less than a third of all light-for-dates infants, and less than a third of pregnancies with low estriol values were associated with births of light-for-dates infants. Our reanalysis of data from three other published studies, using the same method of assessment, gave similar results. When laboratory tests are expected to provide clinically relevant information, we urge that data should be appropriately collected, analyzed, and reported, so clinical usefulness can be evaluated.

Additional Keyphrases: *fetal status* · *screening* · *estrogens* *monitoring pregnancy*

Measurements of estriol in the mother's serum are widely used for assessing fetal wellbeing. For such measurements to be useful as a screening test they should predict the birth of at-risk infants. There have been contrasting opinions expressed in the literature on the usefulness of estriol measurements. However, in many such studies the data were not analyzed appropriately to evaluate whether the test contributes useful information for making clinical decisions. In this study we prospectively investigated the usefulness of estriol measurements, taking regard of the clinical context.

Methods

The subjects in this study were women whose pregnancies were reliably dated to be at 35–36 weeks of gestation by ultrasound examination or certain menstrual history and thorough clinical assessment. They provided plasma samples (n = 362) and 24-h urine collections (n = 356). The specimens were taken prospectively from the women when they attended the antenatal clinic, and each woman provided only one blood and urine specimen. One-third of the women were primigravidae; twin pregnancies were excluded. Plasma unconjugated (*1*) and total estriol (Oestriol Total Kit; Radiochemical Centre, Amersham, U.K.) were measured by RIA, and 24-h urinary estrogen was determined by fluorometry (*2*). After the babies were born their weights were noted. Infants with birth weights below the tenth centile of data derived from a South Island, New Zealand, population (*3*) were defined as being of low birth weight for gestational age, or "light for dates."

Results

We calculated the means and standard errors of each of the estriol fractions for both the women with low-birth-weight babies and those with normal-birth-weight infants. The mean of the estriol values was lower in the women with low-weight babies for all three fractions measured (Figure 1) with statistical significance by *t*-test (urine estrogen $p < 0.001$, plasma total estriol $p < 0.05$, plasma unconjugated estriol $p < 0.02$). Although the means were different, there was some overlap between the groups of women with normal- or low-weight babies.

Because of the imperfect separation of the groups two indices of diagnostic efficiency of the tests were defined: "sensitivity" as "the fraction of mothers carrying low-weight babies who had estriol concentrations below a specified centile," and "specificity" as "the fraction of mothers with estriol concentrations below a specified centile, who had low-weight babies."

Table 1 gives the sensitivities and specificities for the three estriol fractions that were measured, for two cutoff levels. The analysis that was performed enabled convenient and simple comparison of clinical usefulness at different cutoffs; e.g., use of the twentieth rather than the tenth centile of urinary estrogen excretion increased the number of low-weight babies identified (sensitivity), but at the same time decreased the proportion of those babies in the whole cutoff group (specificity). Because of contradictory state-

Table 1. Sensitivities and Specificities Calculated at Two Different Cutoffs, Demonstrating the Ease of Comparing Diagnostic Efficiencies at Different Cutoffs

	Sensitivity	Specificity
Urinary estrogen		
10th centile as cutoff	0.32	0.31
20th centile as cutoff	0.50	0.26
Plasma total estriol		
10th centile as cutoff	0.22	0.23
20th centile as cutoff	0.48	0.24
Plasma unconjugated estriol		
10th centile as cutoff	0.21	0.22
20th centile as cutoff	0.31	0.17

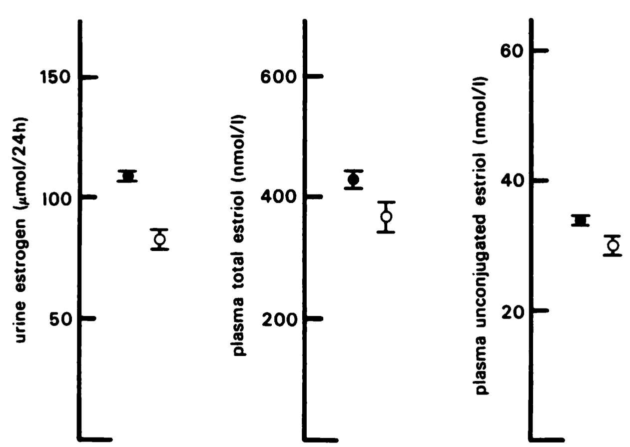

Fig. 1. Mean ± SEM of values for pregnant women
On the *left* of each panel is the value for mothers with normal-weight babies (●), and on the *right* that for those with low-weight babies (○)

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ments in the literature on the usefulness of estriol for detecting light-for-dates fetuses, we reanalyzed published data by the method described. Sufficient information was identified from three studies to calculate sensitivities and specificities (Table 2). In each study the proportion of all light-for-dates births correctly identified (a third or less) was comparable with our own results, as was the proportion of falsely identified normal-for-dates births (two-thirds or more of those below the chosen cutoff).

Discussion

Deficiencies of previous studies addressing a similar problem to that in the present investigation included poorly defined end-points and combining different pathophysiological conditions. In this investigation there was an objective measure, i.e., low birth weight for gestational age, a condition known to be associated with perinatal problems.

Table 2. Examples of Sensitivities and Specificities Calculated from Data Published by Other Workers, Illustrating the Convenience and Usefulness of the Definitions for Evaluating Diagnostic Efficiency

(*i*)[a] urine estrogen (uE) (*15*)
used tenth centile (10c) of uE as cutoff
(stillbirths)+(neonatal deaths)+(birthweights <10c)
with normal uE = 1647
with uE <10c = 889

$$\textit{sensitivity} = \frac{889}{1647 + 889} = 0.35$$

total babies delivered	=	31 263
number with birthweight <10c	=	3126

$$\textit{specificity} = \frac{889}{3126} = 0.28$$

(*ii*)[b] plasma total estriol (pTE_3) (*9*)
used tenth centile (10c) of pTE_3 as cutoff
small for gestational age

total	=	23
with pTE_3 <10c	=	6

$$\textit{sensitivity} = \frac{6}{23} = 0.26$$

total number with pTE_3 <10c	=	28

$$\textit{specificity} = \frac{6}{28} = 0.21$$

(*iii*)[c] plasma total estriol (pTE_3) (*6*)
used 1 SD of pTE_3 as cutoff

percent IUGR babies >1 SD below mean	=	33%
sensitivity = 0.33		
total no. assayed >90+13+8+8	=	119
number with pTE_3 >1 SD below mean = 16% × 119	=	19
total no. with IUGR assayed	=	15
no. with IUGR and pTE_3 >1 SD below mean = 33% × 15	=	5

$$\textit{specificity} = \frac{5}{19} = 0.26$$

(*iv*)[c] plasma unconjugated estriol (pUE_3) (*6*)
used 1 SD as cutoff

percent IUGR babies >1 SD below mean	=	22%
sensitivity = 0.22		
total no. assayed >76+11+7+8	=	102
no. with pUE_3 >1 SD below mean = 16% × 102	=	16
total no. with IUGR assayed	=	14
no. with IUGR and pUE_3 >1 SD below mean = 22% × 14	=	3

$$\textit{specificity} = \frac{3}{16} = 0.19$$

[a] data mainly from reference *15*, table III; [b] data mainly from reference *9*, table III; [c] data mainly from reference *6*, table I and figure 2.

Some workers have commented favorably on the usefulness of estriol measurements in pregnancy as a screening test (*4–6*). In contrast, others have suggested that rather than as a means of identifying an at-risk population, estriol is chiefly useful for reassurance (*7–9*). (However, a test with a cutoff at the tenth centile of any random variable will provide correct reassurance at least eight of nine times when growth is satisfactory.) Still other workers have had doubts about the usefulness of estriol assay for screening (*10–12*). In these published studies, rarely were the data definitively analyzed in the context of clinical usefulness.

The results of the present study demonstrated the ability of values for estriol to mathematically distinguish an at-risk group from the normal population. Nevertheless, after defining two complementary indices with useful clinical relevance, using a cutoff at the tenth centile—a level frequently chosen—the study demonstrated that the calculation of sensitivity revealed that less than a third of mothers with low-birth-weight babies also had low values for estriol, and calculation of specificity revealed that less than one-third of mothers with low estriol values had low-weight babies. The results of other workers that we analyzed using the defined indices gave values of similar magnitude, allowing for different cutoff values and different protocols.

The method described here for analyzing the data is simple and is immediately interpretable in the clinical context. Sensitivity provides information on the fraction of the defined at-risk group that is detected by a test, and specificity provides information on the extent to which a test distinguishes the at-risk group from the whole population. It is of prime importance to collect and analyze data appropriately when the usefulness of a test is being evaluated.

Estriol secreted by the fetoplacental unit is metabolized in the maternal compartment. Estriol probably is metabolized to different degrees by different women (*13, 14*). Therefore information on estriol concentration will perhaps be of limited use as an antenatal screening test of unselected pregnancies, as indicated by the rather high false-positive and false-negative prediction rates found in the reported studies, including our own. However, estriol measurement is likely to remain a useful test for monitoring the course of a pregnancy, where trends can be observed in conjunction with absolute values, when repeated sampling can be clinically and economically justified.

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Improved Liquid-Chromatographic Determination of 3-Methoxy-4-hydroxyphenylethyleneglycol in Urine with Electrochemical Detection

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In this improved method for quantifying 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG) in urine, after a multistep extraction of MHPG and internal standard (iso-MHPG) from 3.0 mL of urine, the compounds are separated on a C_{18} reversed-phase column and quantified by use of an electrochemical detector. The isocratic chromatographic separation takes about 16 min. The mobile phase is phosphate buffer/acetonitrile (88/12 by vol), the flow rate 0.7 mL/min. Recycling the mobile phase and automating the sample injection make possible the unattended assay of more than 70 samples per day. The within-run precision of the method is excellent (CV 1.8%) at a mean concentration of 1.1 mg/L.

Additional Keyphrases: *gas chromatography/mass spectrometry · tricyclic antidepressants*

3-Methoxy-4-hydroxyphenylethyleneglycol (MHPG) is the major metabolite of norepinephrine in the central nervous system (*1*). In human urine, MHPG is mainly present in the form of sulfate and β-glucuronide conjugates, with only a small proportion in the nonconjugated form (*2, 3*). Since the early 1970s, several research groups have studied the rate of excretion of MHPG and its relationship to depressive disorders (*4*). Information on MHPG concentrations is now widely used to predict which tricyclic antidepressant drug—in particular, imipramine or maprotiline—will produce the most favorable clinical response (*5*). Our goal was to provide a reliable assay for in-house use so that such clinical decisions could be made relatively quickly as compared with time required for an externally done assay.

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In most of the early clinical studies, urinary MHPG was measured by gas–liquid chromatography (GLC) with electron capture detection (for a review, see *6*). More recent modifications of this method are still time-consuming, requiring over 3 h of instrument time per sample (*7*), and results correlate only modestly (r = 0.61) with a gas chromatographic/mass spectrometric reference method (*6*). For these reasons we chose to investigate liquid chromatography.

Several reversed-phase liquid-chromatographic procedures for urinary MHPG have been described, involving spectrophotometric (*8*), fluorescence (*9*), and electrochemical detection (*2, 10–12*). One might conclude that the liquid-chromatographic methodology has been well established, but there are significant shortcomings. Several of the reports involve screening procedures for catecholamine-secreting tumors, where there is little concern for precision and exact quantitative measurements (*2, 10*). Most of the others lack sufficient experimental detail to allow duplication of their results, present no data on precision, and do not include a suitable internal standard.

The present assay, based on our investigation of the two most reliable methods, those developed by Moleman and Borstrok (*11*) and Santagostino et al. (*12*), incorporates the best features of each method, with modifications to increase sensitivity and precision. We describe in detail the extraction procedure. The equipment required is relatively simple and inexpensive. We believe that the present procedure is suited for widespread use in the routine clinical laboratory.

Materials and Methods

Apparatus

We used a Model LC-150 "high-performance" liquid chromatograph equipped with an electrochemical detector con-