Laboratory Assessment of Diabetic Pregnancy

A Brief Review

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SUMMARY

Pregnancy in the diabetic is at high risk. Recent efforts to improve perinatal mortality have principally been directed at establishing a laboratory assessment which could be used in the management of the diabetic pregnancy. Data on the hormones—estrogen, progesterone, human placental lactogen (HPL) and human chorionic gonadotropin (HCG) and on the enzymes—heat-stable alkaline phosphatase (HSAP) and diamine oxidase (DAO) are briefly reviewed for uncomplicated and for diabetic pregnancies. DIABETES *21*: 31-38, January, 1972.

Pregnancy complicated by diabetes mellitus is unequivocally at high risk. Prior to the insulin era such pregnancies, which rarely occurred at the time, were almost always unsuccessful. By 1940, however, the over-all perinatal mortality had been reduced to 40 per cent and by 1960 to 15 per cent.[1,2] The expected over-all perinatal mortality in the general obstetric population is about 3 per cent.[3]

The reduction of perinatal mortality to 15 per cent is largely due to improved diabetic control, earlier timing of delivery, excellent care of the newborn and the one-team approach to the management of the diabetic pregnancy.[1,4-6] During the last ten years, little additional improvement in the perinatal mortality has been achieved, however. Perinatal mortality statistics continue to range from about 10 per cent[2,7] to about 20 per cent.[8-10] Prematurity by both gestational age and birth weight is a more important factor in the over-all perinatal mortality than fetal death in utero.[4]

Although the clinical assessment of diabetic pregnancy remains essential, attempts during the past decade to reduce further perinatal mortality have largely been directed at a laboratory assessment which might indicate the potential success or failure of the pregnancy. When such tests have been used in the management of high risk pregnancies, including those in diabetics, they have been applied with the hope that if the results suggested that fetal death in utero was imminent, intervention by immediate delivery would be essential. On the other hand, if such tests indicated normal physiology, delivery of that high risk pregnancy might be postponed to lessen the degree of prematurity. Our experience indicates that this latter point is the most critical one. Thus, the ideal delivery time is the moment, in any given high risk pregnancy, which minimizes both death in utero and the degree of prematurity.

The establishment of a baseline laboratory assessment and its application to the prediction of the outcome of both diabetic and other high risk pregnancies have been determined in a variety of hormonal, enzymatic, and cytologic studies. Several reviews on various aspects of this subject are available.[11-20] In most instances either conflicting results have been obtained, or the data have been too few to be little more than suggestive. In most studies, and particularly in those involving diabetes mellitus, limited numbers of patients have been used. Clearly, the laboratory profile of any one or more tests which will help establish the ideal time for delivery in high risk pregnancies including diabetes mellitus has not been established.

Hormonal studies on estrogen, progesterone, human chorionic gonadotropin and human placental lactogen and enzymatic studies on heat-stable alkaline phosphatase and diamine oxidase have been most often studied and are of particular interest. The potential of each of these for uncomplicated and for diabetic pregnancies will be discussed in more detail.

ESTROGEN

Of all of the laboratory studies related to the welfare

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of a high risk pregnancy, those on estrogen are certainly the most common. A recent excellent review of the subject is available.[21] Estriol, the most abundant estrogen, more directly represents the function of both the fetus and the placenta (the fetoplacental unit) while estrone and estradiol-17B more closely portray placental function alone.[19,22,23] Although not completely accepted,[21] estriol levels more closely correlate with fetal weight[10,24,25] than placental weight.[10] Such data are not available for estrone and estradiol-17B.

Urinary estriol has been the hormone most frequently studied. Relatively little data are available on urinary estrone and estradiol-17B. Technics for such studies are reasonably simple, accurate, and allow handling of multiple samples.[24] Several problems exist, however, which include wide daily variability,[21,24] requirement of cumbersome twenty-four hour urinary collections,[26] and the influences of posture,[27] changes in renal function,[28,29] and the presence or absence of glycosuria.[30] In uncomplicated pregnancies very low levels of urinary estriol can be detected before the sixth week of gestation. A steady rise in mean twenty-four hour urinary values from 4.4 mg. at sixteen to twenty weeks to 26.1 mg. at term occurs.[21,31] The lower limit of normal range rises from 2 mg. at sixteen to twenty weeks to 12 mg. at term.[21,31] Although most investigators feel that twenty-four hour urinary estriol excretion in the diabetic pregnancies falls either below or in the low normal range,[7,24,26,32-35] some have found no difference, and Easterling et al.[10] found higher than normal values.

It is generally agreed that beyond the thirty-fourth week of gestation a twenty-four hour urinary estriol excretion greater than 12 mg. is associated with a successful outcome. A value below 4 mg. indicates fetal death has occurred or is imminent. Isolated twenty-four hour urinary estriol values between 4 and 12 mg. suggest fetoplacental jeopardy and become increasingly more meaningful when serial studies indicate a falling value.[24] Most[7,9,10,25,26,31,32,35-37] although not all investigators[38,39] find serial twenty-four hour urinary estriol values useful in managing most high risk pregnancies including diabetes mellitus.

More recently the determination of plasma estriol has been recommended as a laboratory test superior to that of urinary estriol.[21,26,40-42] The advantages of plasma over urinary estriol include ease of sampling, less influence of posture and renal function, lack of influence by hyperglycemia, and short half-life of probably less than two hours allowing for more than a single sample per day.[21,26,40,42-44]

Because of daily variability, serial studies of plasma estriol are required.[45] Only a limited number of studies are available, however, on plasma estriol. Ratanasopa et al.[26] have shown that normal uncomplicated pregnant females have a range of plasma estriol of 0.5 μg. per cent to 3.0 μg. per cent at twenty-five weeks gestation which steadily rises to 9.0 μg. per cent to 22 μg. per cent at term. Nachtigall et al.[42] have shown a similar pattern with a range of 9.3-56 μg. per cent (mean, 27 μg. per cent) at term. In the diabetic pregnancy Nachtigall et al.[40] found no difference between normal and well controlled Class A, B or C diabetic pregnancies (White Classification—see reference 2). Levitz et al.[41] from the same group later reported higher than control plasma estriol levels in Class D diabetic pregnancies.

The preliminary data on plasma estriol, like those of urinary estriol, indicate that a falling value, particularly below 9.0 μg. per cent, might indicate that the pregnancy is in jeopardy. Values below 2.6 μg. per cent appear to indicate fetal death.[40] In contrast to Classes A, B and C, Levitz et al.[41] failed to find plasma estriol useful in following Class D diabetic pregnancies.

HUMAN PLACENTAL LACTOGEN (HPL)

Undoubtedly one of the most promising developments in the field of obstetrics and endocrinology has been the discovery and characterization of HPL-chorionic growth hormone-prolactin,[46] purified placental protein,[47] or human placental lactogen (HPL).[48] HPL will be the term used throughout this discussion. The chemical, immunological and biological characteristics of HPL have been described. Several recent reviews are available.[46,49-52]

HPL is produced exclusively by placental syntrophoblastic cells and secreted almost entirely into the maternal circulation in small amounts early in pregnancy with progressively increasing quantities to term.[48,53-56] HPL levels have been found by many[57-60] although not all investigators[61,62] to correlate positively with placental weight but not fetal weight.[60] HPL can be measured rapidly and accurately by radioimmunoassay of small samples of plasma or serum, refrigerated for up to forty-eight hours.[53,54,60-64] HPL appears to be a hormone present in reasonably large amounts with a very short biologic half-life of less than thirty minutes and without a circadian rhythm, which is unaffected by physical activity, state of nutrition, or level of blood glucose.[56,61] Urinary levels of HPL have found little favor since they represent only a fraction of serum levels, vary with renal hemodynamics, and require cumbersome sample collection.[46]

Since maternal HPL appears to represent closely the

secretory functional mass of the placenta and methodologically is almost an ideal substance to measure, an increasing number of serial studies in normal and complicated pregnancies have appeared during the last few years. Particular attention has been paid to the potential usefulness of HPL as an important prognostic tool in the management of high risk pregnancies.

Several studies in uncomplicated pregnancies are available.[56,58,60,65] Lack of standardization of the immunoassay technic between laboratories has limited comparison of some but not all of the data.[66] Serum HPL has been detected as early as the fifth week of gestation[60,67] with values of 1 mμg./ml. A steady increase in serum HPL occurs until the thirty-sixth week with control values between 5 and 10 μg./ml.[68] During the last four weeks of pregnancy HPL has been described as reaching a plateau,[11,60,61,65,67] decreasing slightly[58,62,68] or increasing slightly[54] to term. During labor HPL is quite variable and usually lower than just prior to the onset of labor which, if the "term" samples were taken at this time, may explain some of the differences in the curve of HPL during the last four weeks of pregnancy.[11,60,61] This may also explain the lack of correlation of HPL and the placental weight, as noted by some investigators.[61,62] Normal control data for HPL established for our clinic show a steady rise from barely detectable levels at about six weeks to a mean plateau value of 5.7 μg./ml. at thirty-six weeks.[69]

Considerably less data are available concerning HPL in diabetic pregnancies than that available for estriol. Although a great deal of overlap exists, mean serum HPL values have been found to be nearly identical[11,54,58,64] to control values or to be higher[60,62,64,68,69] than control values in diabetic pregnancies. Data have been established at our clinic concerning serial HPL levels in diabetic pregnancies.[69] Both hormone and nonhormone-treated diabetic pregnancies were studied. Serum HPL parallels normal values up to about twenty weeks, then rises more rapidly to a higher than normal level from thirty-six weeks to term.

Several studies are available evaluating the usefulness of HPL in predicting the potential success of high risk pregnancies in general. The numbers of patients are small, however, and the data in most instances preliminary. Nevertheless, serial studies suggest that HPL may well prove to be of benefit in the management of such pregnancies. Serum HPL values which are within the normal range or which may be somewhat low with either a slight rise or consistently level course during the third trimester of pregnancy in general are associated with successful outcome of the pregnancy. Values which show an abrupt or slow but steady decline are more closely associated with an unsuccessful pregnancy. However, a recent panel of authorities caution that the data are very preliminary and that future studies are necessary to determine the usefulness of HPL as a method of predicting the outcome of high risk pregnancies.[66]

Specific data on the application of HPL studies to the management of diabetic pregnancy are very limited and are in conflict. Our data[60] and that of others[62,68] suggest a potential usefulness, while still others[11,58,64] fail to find any correlation with the outcome of the diabetic pregnancy and thus any usefulness.

HUMAN CHORIONIC GONADOTROPIN (HCG)

Human chorionic gonadotropin (HCG) represents another hormone of placental origin principally secreted into the maternal circulation which has been studied in normal and in complicated pregnancies. The bioassay technic performed on urinary samples, which represents less than 20 per cent of placental secretion, has had limited usefulness in the understanding and management of high risk pregnancies.[70-72] Only recently with the successful application of radioimmunoassay technics to serum samples has a resurrection of interest in HCG occurred.

Compared to estriol and HPL, little serial data on immunoassayable serum HCG is available in the literature. Midgley[72] has reviewed his experience. Early in pregnancy a peak level of HCG occurs followed by a second lower peak and then a constant decline to a low plateau level at term. Bruner[71] with a plasma bioassay technic found a similar curve. Samaan et al.[11] by serum immunoassay found a single early peak followed by a constant low level plateau to term. The mean serum peak value at twelve to sixteen weeks gestation was 36 IU./ml. and the nadir plateau mean value was 12 IU./ml.[11] Data obtained for our clinic measuring HCG by a rapid, serum immunoassay technic show appreciable detectable levels to be present at about five weeks. When gonadotropins are being studied serially during the menstrual cycle, serum HCG has been detected as early as the first week after inception.[72B] The serum HCG level rises sharply to a mean peak value of 163 IU./ml. at eight to ten weeks, falls to nadir of 12 IU./ml. at about eighteen weeks and then rises again gradually to a mean plateau value of 63 IU./ml. at about thirty-six weeks.

Only our studies[69] and those of Samaan et al.[11] are available on the radioimmunoassay of serum HCG for the diabetic pregnancy in particular. Samaan et al.[11]

showed no early peak of HCG in these pregnancies. Instead they found a gradual rise from about 15 IU./ml. at twelve weeks to a peak of about 45 IU./ml. at thirty-six weeks gestation with a slight decline to term. Data from our clinic for hormone and nonhormone-treated diabetic pregnancies show a scatter of values greater than that noted in our controls. However, the mean HCG values throughout the entire pregnancy appear to be higher than controls particularly during the last trimester. The mean peak value at eight to ten weeks gestation of 194 IU./ml. falls to a nadir of 67 IU./ml. at about eighteen weeks and then rises rapidly to a higher than control mean plateau value of 135 IU./ml. at about thirty-six weeks. During the early part of diabetic pregnancy a discrepancy exists between our data[69] and those of Samaan et al.[11] General agreement, however, exists on the elevated HCG values during the latter half of the pregnancy.

HCG studies have in general proven useful only early in pregnancy during the peak secretion, particularly as a diagnostic tool for molar pregnancies or threatened abortions.[50,72] Once a pregnancy has progressed beyond the previable date of twenty-eight weeks gestation, most studies have shown no particular benefit of HCG levels in predicting the outcome of the pregnancy principally because of the low values.[12,72-74] The very recent finding that serum values might be elevated in diabetic pregnancies suggest that in this type of high risk pregnancy serial HCG determinations may well prove useful in the management program. Such studies, however, have yet to be carried out.

PROGESTERONE

Progesterone represents a hormone which can be produced de novo from acetate by the placenta,[22] the only important source during the latter half of pregnancy.[75] Most available studies during pregnancy have been concerned with the measurement of its major urinary metabolite, pregnanediol. Mean twenty-four hour urinary pregnanediol excretion in uncomplicated pregnancies rises steadily from very low levels during the first few weeks of pregnancy to 10 μg. at ten weeks gestation and then more rapidly to 45 μg. at thirty-six weeks gestation with a slight decline to term.[49] Urinary pregnanediol studies in diabetic pregnancies are limited. Low[34,77] and normal[11,76] values have been reported.

Several studies[12,49,74,77] have found urinary pregnanediol of limited usefulness while others[11,13,76,78] of no benefit in the management of high risk pregnancies including diabetes. The particular problems include the fact that urinary pregnanediol represents only 10 to 20 per cent of placental progesterone secretion,[79] correlates poorly with serum progesterone[12] and, unless there is significant placental dysfunction, levels can remain quite normal long after fetal death in utero occurs.[11,12,75]

More recently attention has been diverted to studies on serum progesterone which represents a more direct measure of placental function. The data suggest that serum progesterone may be considerably more useful than urinary pregnanediol, but no systematic studies are available in uncomplicated or high risk pregnancies including diabetes. Once again a limiting factor is that intrauterine fetal demise can occur without significant change in serum progesterone levels.[80]

Placental progesterone is in part converted by the fetal adrenal to 17-hydroxyprogesterone which in turn is excreted principally by the mother as urinary pregnanetriol.[13,16,22,79] Thus, 17-hydroxyprogesterone and pregnanetriol represent more closely the fetoplacental unit.[79] Their potential usefulness in following high risk pregnancies has been studied.[13] Acevedo et al.[16] found serial twenty-four hour urinary pregnanetriol to follow a pattern similar to urinary estriol.

HEAT-STABLE ALKALINE PHOSPHATASE (HSAP)

Most all of the readily available serum enzymes of general clinical laboratory usefulness have been studied in pregnancy.[17,81] Alkaline phosphatase appears to show the greatest promise as a measure of the status of the pregnancy.

The heat-stable fraction of circulating alkaline phosphatases is an enzyme of placental syntrophoblastic cell origin.[82-84] Unlike HPL, HSAP levels have not correlated with placental or fetal weight.[85,86] This enzyme has a rather long biologic half-life of about three days and is secreted unidirectionally from the placenta into the maternal circulation.[85,87]

Although some variability exists between studies,[87,88] during the last two trimesters in pregnancy mean serum HSAP levels rise steadily from 2.9 KAU to a peak of 10.1 KAU at term.[85,89,90] Limited studies are available comparing the changes in HSAP during the diabetic pregnancy with those of uncomplicated pregnancies. HSAP has been reported to be low[85,86] or normal[91] in diabetic pregnancies.

Studies of serum HSAP in high risk pregnancies have been made to determine its potential usefulness as a measure of placental function. HSAP does not reflect fetoplacental function as a unit. The data are limited and the results unclear. Those who found any promise in the studies recommend only serial determinations. Falling or persistently low values[15,88,90] rapidly rising

to elevated levels,[85,87] or a fluctuating pattern with an initial fall followed by a rise,[92,93] all have been associated with increasing perinatal mortality. Others have found HSAP of little usefulness particularly because of its long half-life, representation of placental function only, and because of its failure to correlate with the outcome of the pregnancy.[86,91]

DIAMINE OXIDASE (DAO)

Diamine oxidase (DAO) represents another enzyme of placental origin which has been studied considerably less well than HSAP. Of particular interest is its origin from the decidual tissue rather than the syntrophoblast.[94]

Uncomplicated pregnancies show a progressive rise in plasma from unmeasureable levels before the twentieth week of gestation to over 500 U./ml. at term (mean value, 1,027 U./ml.).[15,17,94] DAO has been reported to be low-normal or low in diabetic pregnancies.[17,95] Falling levels or persistently low levels of DAO are most often associated with unsuccessful pregnancy.[15,17,94,95] Obviously the data on DAO show promise of a very preliminary nature.

COMBINATION STUDIES

The potential degree of usefulness for the various hormones and enzymes studied individually in predicting the outcome of high risk diabetic pregnancies have been discussed. Since the various factors may have different sites of origin and may represent different aspects of the maternoplacental and fetoplacental units, the potential of combining two simultaneous measurements has been made in several studies. The data suggest that serial combination studies may be potentially more useful than single determinations. Listed below are the various combined measurements which have been compared and which may be useful:

Urinary pregnanediol—Urinary estriol[73,74]
Urinary pregnanetriol—Urinary estriol[16]
Urinary pregnanediol—Urinary HCG[77]
Urinary estriol—Serum HSAP[85,88]
Urinary estriol—Plasma HPL[57,63]

Data have been collected at our clinic comparing serum HCG and HPL.[69] Three possible patterns are apparent. Pattern I shows an elevated HPL with normal HCG values; Pattern II, elevated levels of both; and Pattern III, elevated HCG with essentially normal HPL levels. Because of the limited number of studies, comparisons of such patterns with the outcome of diabetic pregnancies have not yet been made.

MISCELLANEOUS STUDIES

A variety of other laboratory tests have been carried out in an attempt to separate the potentially successful from unsuccessful high risk pregnancies. Although isolated studies suggest value for some, little data are available to indicate any advantage over the hormone and enzyme studies already presented. Studies on pituitary growth hormone, follicular stimulating hormone (FSH), and luteotrophic hormone (LH) show the values to be low and to remain low during normal and probably during abnormal pregnancies.[11,96] Although total plasma cortisol is elevated secondary to estrogen-induced increased binding protein, free cortisol probably remains normal throughout uncomplicated and most other high risk pregnancies.[14] Elevated serum copper concentration[97] and thyroxine-binding globulin levels[98] are seen in pregnancy and falling values have been used as an index of pregnancy in jeopardy. Like cortisol, they reflect only changes in estrogen metabolism and therefore, add little to the more direct measurement of the hormone. Recently another placental hormone, human chorionic thyrotropin, has been characterized. Serum levels have been found to be elevated during pregnancy but data related to pregnancies at high risk are not available.[99] As already mentioned, a multitude of other circulating serum enzymes of clinical interest have been measured during pregnancy. Possibly with the exception of aminopeptidase (oxytocinase)[100,101] they provide no important information in the evaluation of normal or high risk pregnancies.[17,81]

Cytologic evaluation of vaginal secretions has been useful in early pregnancy but of little use as an index of the status of the pregnancy later in the course.[12,15,74,78] Once again this technic represents an indirect measure of estrogen and progesterone effects on tissues and provides little information over the more direct hormonal measurements. Studies on amniotic fluid for meconium, estriol and other substances have also been used with varying success to follow high risk pregnancies.[102,103] Amniocentesis is a specialized procedure with morbidity greater than venipuncture or urine sampling and at the present time with limited general applicability. These studies have proven to be more useful in the antenatal evaluation of genetic disorders, fetal maturity and Rh incompatibility.

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