Biochemical tests for abnormalities in pregnancy

Huy A. Tran, Head and Associate Professor, Department of Clinical Chemistry, Hunter Area Pathology Service, John Hunter Hospital, Newcastle University, Newcastle, New South Wales

Summary

Pregnancy induces major physiological, hormonal and biochemical changes to achieve an optimal outcome for the baby and its mother. When the pregnancy deviates from its normal course, there are many biochemical markers which can be used to assess these abnormalities. As biochemistry is only one part of obstetric care, results should be interpreted in conjunction with clinical and medical imaging data. Imaging is especially important and can be used to assess many placental and fetal abnormalities. Ultrasonography continues to improve and be refined in the early detection of fetal structural defects. It has equalled, if not superseded, biochemical testing in many aspects of obstetric care.

Key words: alpha fetoprotein, human chorionic gonadotrophin.

(Aust Prescr 2006;29:48–51)

Introduction

Biochemical markers are used to assess maternal, placental and fetal health. They help to diagnose and monitor maternal conditions such as gestational diabetes and pre-eclampsia, trophoblastic disease and fetal chromosomal abnormalities such as Down's syndrome (Table 1). These biochemical and hormonal tests constitute only one aspect of obstetric care. They should be used together with clinical findings and imaging, particularly ultrasonography.

Biochemical assessment of maternal health

Common problems in pregnancy include gestational diabetes and pre-eclampsia.

Diabetes

The prevalence of gestational diabetes mellitus ranges from 1 to 14% depending on the populations studied.[1] In Australia, the prevalence ranges from 5.5 to 8.8%.[2] Screening for gestational diabetes mellitus in Australia is strongly advocated at 26–28 weeks of gestation. This enables early intervention which results in significant improvements in both fetal and maternal outcomes.[3]

Occasionally, the serum glucose is unexpectedly found to be in the diabetic range in the first trimester. By definition, this is gestational diabetes mellitus, but does not distinguish between diabetes that may have preceded or occurred at the same time as pregnancy. The diagnosis can be confirmed by further tests of fasting glucose concentration or a 75 g oral glucose tolerance test. These patients should be reassessed in the postpartum period for evidence of diabetes. The woman's glycated haemoglobin (HbA1c) should be maintained in the normal range or as near normal as possible to ensure optimal fetal outcome.

Pre-eclampsia

Pre-eclampsia occurs typically in the third trimester and affects 4–8% of pregnancies.[4] It constitutes a triad of pregnancy-associated hypertension (that is, there is no pre-existing hypertension), marked proteinuria (greater than 300 mg daily) and pathological oedema. It is thus critical that urinary dipstick testing for protein, which can be fully quantitated if required, is performed at each antenatal visit together with blood pressure measurement and careful examination for oedema. Other findings include rises in serum uric acid (which can antedate the onset of hypertension), urea and creatinine. Low haemoglobin and platelet concentrations are informative if the patient is suspected to have the severe form of pre-eclampsia – haemolysis-elevated liver enzymes-low platelets (HELLP). In the absence of pre-existing pathology, these biochemical parameters should return to normal after delivery.

Biochemical assessment of placental health

Ultrasonography has added another dimension to first trimester obstetric care to such an extent that many traditional biochemical tests have been rendered redundant. Maternal serum human placental lactogen and serum or urinary oestriol concentrations which were previously used extensively in the assessment of placental function, are rarely used nowadays.

Human chorionic gonadotrophin (HCG)

As pregnancy progresses, the patient's hormonal profile continues to evolve with steadily rising concentrations of progesterone and oestrogen. These continue to rise well into the first trimester while concentrations of luteinising hormone and follicle stimulating hormone are low or suppressed. To maintain

Table 1

Biochemical tests for common maternal, placental and fetal conditions

	Condition	Test
Maternal	Gestational diabetes	Glucose screening tests at 24–28 weeks: 50 g challenge test **or** 2-hour 75 g oral glucose tolerance test
	Pre-eclampsia*	1. Urinary protein (by dipstick testing or formal quantitation) 2. Serum uric acid 3. Renal function tests 4. Full blood count (for Hb concentration and platelet count)
Placental	Trophoblastic disease* (hydatidiform mole or choriocarcinoma)	1. HCG 2. Free β-HCG 3. Urinary HCG when indicated
Fetal	Down's syndrome*	Maternal serum alpha fetoprotein, HCG, pregnancy-associated plasma protein-A, and transnuchal ultrasound between 11 and 13 weeks gestation Maternal serum alpha fetoprotein, HCG, pregnancy-associated plasma protein-A, and serum unconjugated oestriol in various combinations between 15 and 18 weeks gestation
	Neural tube defects	1. Maternal serum alpha fetoprotein **or** 2. Amniotic fluid alpha fetoprotein (less common)

* potential use of fetal DNA in maternal circulation
HCG human chorionic gonadotrophin

progesterone production from the corpus luteum in order to keep the pregnancy viable in its early stage, the placenta starts to secrete HCG. The serum HCG concentration is therefore the test of choice for confirming pregnancy.

Physiologically, serum HCG arising from trophoblastic activity is elevated as early as the eighth day after implantation. Concentrations double every 2–3 days and peak at approximately 10 weeks. They then decline and plateau out at a lower concentration until parturition (Fig. 1).

In addition to confirming pregnancy, serum HCG can be used as a marker to assess various abnormalities in the first trimester. A failure to rise at the appropriate rate suggests the impending loss of the pregnancy from spontaneous miscarriage or an unviable/ectopic pregnancy. A markedly elevated serum HCG suggests the presence of multiple pregnancies, especially with assisted fertilisation, or the presence of gestational trophoblastic disease including chorionic carcinoma and hydatidiform mole. A hydatidiform mole typically appears as a 'snow storm' on ultrasound. Confirmatory biochemical tests should include the **free** β-HCG concentration because this form of HCG is secreted in disproportionately high amounts.[5]

HCG can be used to assess the effectiveness of therapy and monitor for recurrence following surgery for gestational trophoblastic disease. A rapid decline or the disappearance of serum HCG is to be expected after successful surgery. False positive results at low HCG concentrations have been reported and have led to unnecessary surgery.[6] It is therefore important that when the HCG concentration is contrary to the clinical assessment, parallel **urinary** HCG concentrations should be analysed. (If the serum concentration of HCG is low but detectable in a clinically cured patient, the absence of urinary HCG raises the suspicion of a false positive serum result. If HCG is also present in the urine a residual tumour is more likely.) In these complex situations, ongoing communication with the laboratory is critical to the care and outcome for patients.

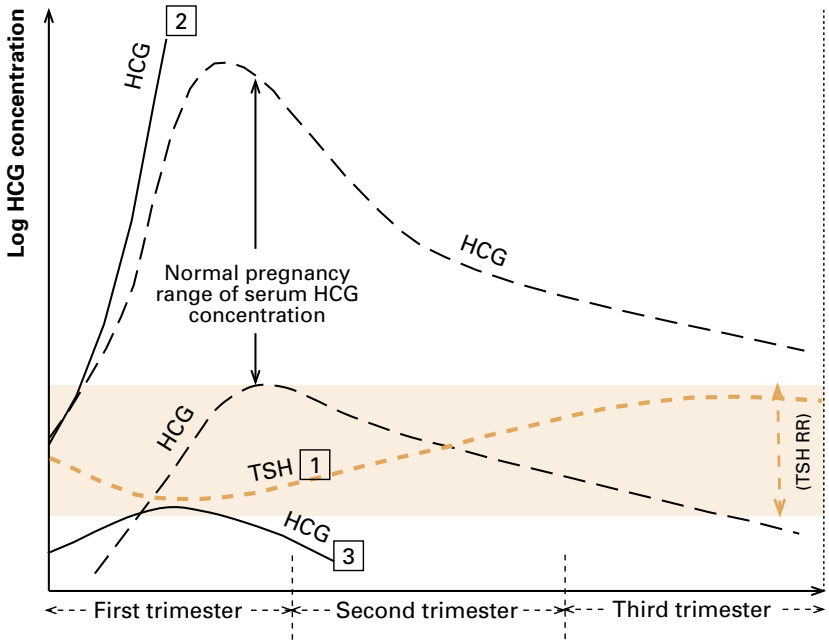
In the second trimester an elevated serum HCG concentration has been associated with a two- to threefold increased risk of fetal growth retardation.[7]

Biochemical assessment of fetal health

The major aim of fetal assessment is to ensure satisfactory growth *in utero*. There are many factors which can cause fetal

Fig.1

Human chorionic gonadotrophin (HCG) and thyroid stimulating hormone (TSH) concentrations



Note the broad reference intervals for HCG which peaks during the first trimester of pregnancy.

1 Represents the normal response of serum thyroid stimulating hormone (TSH) during pregnancy. The reference range (RR) for TSH is in the shaded area. Note the drop in TSH concentration in the first trimester corresponding with the peak HCG concentration. This is due to negative feedback on TSH secretion resulting from the stimulating effect of HCG on TSH receptors.

2 The high baseline HCG concentration with markedly shortened doubling time suggests multiple pregnancy or a trophoblastic tumour.

3 Failure of HCG to rise or double with time. This suggests the presence of an unviable or ectopic pregnancy or threatened miscarriage.

growth retardation. These range from poor maternal nutritional state to placental insufficiency and fetal abnormality. Similar to placental function, medical imaging is increasingly used to detect fetal abnormalities, thus reducing the utility of biochemical markers.

Alpha fetoprotein

Alpha fetoprotein is a fetal protein arising from the yolk sac and fetal liver. It can be detected in increasing concentrations in maternal serum until 32 weeks of normal gestation.

Neural tube defects

In neural tube defects such as spina bifida[8] and anencephaly, the concentration of alpha fetoprotein in the maternal serum is unusually high in the first trimester because cerebrospinal fluid leaks into the amniotic fluid. Other causes of elevated alpha fetoprotein, such as incorrect gestational date and multiple pregnancy, need to be excluded. As a marker of neural tube defects maternal serum alpha fetoprotein, ideally, should be measured between 15 and 18 weeks of gestation. Any suspicion of a neural tube defect can be further assessed with ultrasound, usually at 18–20 weeks. This scan also assesses for other fetal morphological abnormalities and placental placement.

Down's syndrome

Down's syndrome is one of the common causes of fetal growth retardation. It is the result of either partial or total trisomy of chromosome 21 and is a major obstetric concern, particularly in older women. Important biochemical markers include alpha fetoprotein, HCG, unconjugated oestriol, pregnancy-associated plasma protein-A, serum inhibin-A and free β-HCG. These markers are used in various combinations and together with ultrasound to increase the detection rate of Down's syndrome. It cannot be overemphasised that the gestational age must be correct in order for screening parameters to be accurate.

Between 11 and 13 weeks (that is late **first** trimester), serum

pregnancy-associated plasma protein-A, free β-HCG and ultrasound assessment of nuchal thickness (the physiological space between the back of the neck and the overlying skin of the fetus) are most commonly used in the assessment of Down's syndrome. Due to the changing concentrations of these markers in the normal pregnant population, the results are mathematically corrected for easy comparison. The nuchal thickness is increased in Down's syndrome and approximately 70% of cases will be detected by ultrasound in experienced centres. In combination with biochemical markers, the detection rate increases to 85–90%.[9,10] Abnormal results can be followed up with direct karyotyping using chorionic villous sampling, but this carries a 0.5–1.0% risk of pregnancy loss in the first trimester.

In the **second** trimester, screening for Down's syndrome traditionally employs the triple test of maternal serum HCG, serum unconjugated oestriol and alpha fetoprotein at 15–18 weeks of gestation. Some laboratories also measure serum pregnancy-associated plasma protein-A. The combination of these markers and maternal age delivers a 60–65% detection rate, but this includes the 5% of women who have a false positive result. Transnuchal thickness in the mid to late second trimester does not correlate well with Down's syndrome and does not add to the value of biochemical markers.[11]

The results of Down's syndrome screening in the first and second trimester are expressed as the proportion of affected pregnancies, for example 1 in 488 chance of having Down's syndrome. This is accomplished using a risk-assessment program that incorporates nuchal thickness (only in the first trimester), biochemistry results and maternal age.

Other approaches

Another biochemical method of assessing fetal health is the analysis of amniotic fluid. The measurement of bilirubin concentration in amniotic fluid is critical for assessing fetal intravascular haemolysis in the presence of Rhesus incompatability. The lecithin-to-sphingomyelin ratio in amniotic fluid can be used to assess fetal lung maturity in preterm labour but is rarely used these days due to the widespread availability of synthetic surfactant.

Recently, there has been a resurgence of interest using maternal growth hormone and insulin-like growth factor levels during the first and second trimester of pregnancy as predictors of fetal outcome, but these are yet to be of routine clinical use.[12]

Fetal DNA

A major advance in molecular biology has been the possible detection and isolation of fetal DNA in the maternal circulation.[13] This exciting discovery has opened up new horizons in the 'non-invasive' assessment of fetal-maternal health. High concentrations of fetal DNA in the maternal circulation have been found in Down's syndrome, pre-eclampsia, invasive placenta and preterm labour. This technique has also allowed for the prenatal non-invasive diagnosis of Rhesus D genotype, myotonic dystrophy and achondroplasia.[14]

Conclusion

Biochemical markers are important in the assessment of maternal, placental and fetal health. They remain critical in supporting and diagnosing many associated conditions despite the increasing quality and use of ultrasonography. As normal values continue to change with gestational age, these markers should be measured at the correct gestational age to enable accurate interpretation.

References

1. Diagnosis and classification of diabetes mellitus. American Diabetes Association Position Statement. Diabetes Care 2005;28:S37-S42.
2. Hoffman L, Nolan C, Wilson JD, Oats JJ, Simmons D. Gestational diabetes mellitus – management guidelines. The Australasian Diabetes in Pregnancy Society. Med J Aust 1998;169:93-7.
3. Crowther CA, Hiller JE, Moss JR, McPhee AJ, Jeffries WS, Robinson JS. Effect of treatment of gestational diabetes mellitus on pregnancy outcomes. N Engl J Med 2005;352:2477-86.
4. Lyell DJ, Lambert-Messerlian GM, Giudice LC. Prenatal screening, epidemiology, diagnosis, and management of preeclampsia. Clin Lab Med 2003;23:413-42.
5. Cole LA. Immunoassay of human chorionic gonadotropin, its free subunits, and metabolites. Clin Chem 1997;43:2233-43.
6. Rotmensch S, Cole LA. False diagnosis and needless therapy of presumed malignant disease in women with false-positive human chorionic gonadotropin concentrations. Lancet 2000;355:712-5.
7. Onderoglu LS, Kabukcu A. Elevated second trimester human chorionic gonadotropin level associated with adverse pregnancy outcome. Int J Gynaecol Obstet 1997;56:245-9.
8. Mitchell LE, Adzick NS, Melchionne J, Pasquariello PS, Sutton LN, Whitehead AS. Spina bifida. Lancet 2004;364: 1885-95.
9. Roizen NJ, Patterson D. Down's syndrome. Lancet 2003;361: 1281-9.
10. Nicolaides KH. Nuchal translucency and other first-trimester sonographic markers of chromosomal abnormalities. Am J Obstet Gynecol 2004;191:45-67.
11. Cuckle H. Integrating antenatal Down's syndrome screening. Curr Opin Obstet Gynecol 2001;13:175-81.
12. Reis F, D'Antona D, Petraglia F. Predictive value of hormone measurements in maternal and fetal complications of pregnancy. Endocr Rev 2002;23:230-57.
13. Lo YD, Corbetta N, Chamberlain PF, Rai V, Sargent IL, Redman CW, et al. Presence of fetal DNA in maternal plasma and serum. Lancet 1997;350:485-7.
14. Simpson JL, Bischoff F. Cell-free fetal DNA in maternal blood: evolving clinical applications. JAMA 2004;291:1135-7.

Further reading

Joint HGSA/RANZCOG recommended 'Best practice' guidelines on antenatal screening for Down's syndrome and other fetal aneuploidy. 2004. http://www.ranzcog.edu.au/publications/collegestatements [cited 2006 Mar 8]

Conflict of interest: none declared

Self-test questions

The following statements are either true or false (answers on page 55)

7. Alpha fetoprotein is not found in maternal serum during the first trimester of a normal pregnancy.
8. Fetal growth retardation is best assessed by serial measurements of serum oestriol.

Dental notes

Prepared by Dr M. McCullough of the Australian Dental Association

Managing hepatitis C in the community (p.36)

The number of Australian adults living with hepatitis C is increasing and is not confined to any one section of the population. Dentists need to be aware that hepatitis C may be present in the saliva of infected patients. Our infection control practices therefore need to be exemplary to avoid spread of this, and other blood-borne viruses. Dentists are in a position to support medical advice that infected patients undergo antiviral treatment where appropriate and address secondary factors associated with liver disease in these patients.

Any dentists who carry a blood-borne virus have a professional and ethical responsibility to review the way they practise so as to ensure that they minimise the likelihood of infecting their patients. The Australian Dental Association offers advice and co-operation that should be sought.

Your questions to the PBAC

Adrenaline: shelf-life

I was very interested in 'Your questions to the PBAC: Adrenaline' (Aust Prescr 2005;28:90). In particular I wish to comment about the short expiry date of EpiPens.

About six or seven years ago I contacted the distributor of the EpiPen in Australia. I complained that sometimes I would purchase an EpiPen for my son and often it only had seven or eight months left before it expired.

Their explanation was that it was actually transported from the USA and by the time it arrived here many months of its 12-month shelf-life were gone.

On hearing this I checked out an old Martindale (26th edition) and I read that adrenaline in solution was very stable for a number of years. I wrote to the manufacturer of EpiPens in the USA with a photocopy of the extract out of Martindale but never received a reply.

Being a sceptic I just wonder whether it suits the manufacturer to overlook these details as obviously it would affect their sales substantially. Also I think it would be unlikely that a company would actively pursue ways of extending the expiry date!

At the time I was thinking about having the adrenaline stability checked out in an expired EpiPen, but did not have time to pursue this further.

Perhaps if the Pharmaceutical Benefits Advisory Committee (PBAC) did it on a more authoritative basis one might receive a reply.

Kevin Dallimore
Dermatologist
Perth

PBAC response:

The PBAC is aware of the short expiry date of EpiPen. However, the sponsor, CSL Limited, has advised recently that the most recent data from the manufacturer's stability program do not support an extension of shelf-life.

CSL Limited is currently implementing a number of changes to the distribution process. These aim to improve the shelf-life in Australia of EpiPen which is produced with a 20-month shelf-life by the US supplier, Dey Laboratories. The company advises that the following changes have been introduced to minimise the time lost between manufacture and patient in the distribution chain:

- EpiPen will now be produced with Australian packaging by Dey Laboratories to save on repacking time in Australia
- CSL will work with wholesalers and pharmacies to minimise the time stock spends on shelves