Hormone and Enzyme Testing in Pregnancy

Current Research in Obstetrics and Gynaecology

Discussed by Mr Geoffrey Chamberlain and Professor Tim Chard, St Bartholomew's Hospital.

University of London Audio-Visual Centre, 1979.
Made for the British Postgraduate Medical Federation in association with the Blair Bell Research Society.

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Produced by Jennie Smith.

Black-and-white
Duration: 00:34:29:04

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<Opening titles>

<Mr Chamberlain to camera>

Welcome back to the obstetrical and gynaecological part of the University of London's Audio-Visual Centre's programmes. You'll remember in the past seasons, we've had programmes on journal club and then we had a series on dialogues and this season, we're starting a new experiment: we're going to talk about new areas in research. To do this, the University of London Audio-Visual Centre has cooperated with the Blair Bell Research Society, which is the obstetrical and gynaecological society looking at reproductive research in the country, and through them, we're having members of the Blair Bell Research Society come and talk to us about areas

of research that we think will be of interest to you. These will obviously include some of what you know already – I hope so because one always takes off from one's present knowledge – and will include things of interest for research happening now and in the future. And it's on those lines that we hope to run these programmes.

Well, we're starting the first programme, this time, with Professor Tim Chard of St Bartholomew's Hospital and he's going to talk to us on enzyme and hormone testing in pregnancy. Professor Chard.

<Professor Chard to camera>

We have three principal ways of testing the well being of the foetus in a late pregnancy and these can be broadly defined as clinical, electronic and biochemical. The clinical approach is all that traditionally happens in an antenatal clinic under the broadest heading of the laying on of hands, the testing of urine, measurement of blood pressure and the taking of histories. The electronic approach, one of the two principal new ones, involves ultrasound for objective measurement of the size and growth of the foetus in late pregnancy and also for the continuous monitoring by electronic means of the foetal heart during labour. And finally, we have the biochemical approach. Now, the biochemical approach has as its basic principle, the measurement in an accessible site in the mother, in other words, usually blood or urine, of some biochemical product of the foetus or placenta which can be distinguished either quantitatively or qualitatively from the products which are normally present in the non-pregnant adult, and thereby hopefully to identify the overall function of the foetus and placenta.

<Chard refers to chart, on display stand next to him, listing products of the human foetoplacental unit >

Our first picture shows the principal specific products of the human foetoplacental unit. These include certain specific enzymes: heat-stable alkaline phosphatase, cystine aminopeptidase. They include steroid hormone, principally, oestrogens and progesterone. They include protein hormones, all of which are analogues, in other

words, not quite identical, to pituitary hormones in the normal adult and, of course, includes HCG – chorionic gonadotrophin, commonly used as a test of early pregnancy, and HPL, or human placental lactogen, commonly used as a monitor in late pregnancy, and we shall be returning to this material later. There are certain rather recondite materials such as analogues of the releasing hormones and finally, there is a whole new range which I've called here other proteins and I have put up, perhaps, the two most familiar called SP1 and PP5, but there are at least six others. These are specific proteins of the human placenta whose function at the present time is totally unknown.

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<Chard to camera>

Because the title of this programme included the term enzymes, I should perhaps say a brief word, even though it is one of dismissal, about the enzymes. In the sense that they are specific to the placenta, they could operate as tests of placental function. However, they have been extensively tried and I think it is reasonable to conclude have been found wanting. Today, I want principally to address the oestrogens, human placental lactogen and later a brief word about some of the newer placental proteins.

Superficially, the use of these tests should be very straight forward. We measure a given material in maternal blood. If the levels are normal, we say the pregnancy is normal. If the levels are low, we conclude that that foetoplacental unit is not operating at its maximum potential and describe the pregnancy as abnormal. However, that of course is a gross oversimplification of what, in fact, can become a very complex subject. There are many problems.

Now, the first of these problems is a biological one which is when we measure the levels of any of these materials: what is it that we are measuring? We are measuring the ability of the placenta and / or foetus to synthesise a given material, yet we have no guarantee that that synthesis reflects any significant or useful function of the

foetus or placenta. We don't know that a pregnancy without oestrogens or without HPL would not be just as good functionally as a pregnancy with them. What we really want, and I'll address this a little later on, is a test of placental transfer because the transfer functions, the movement of nutrients to the foetus and the waste products from it, are the functions that we really would like to attack. And worse than that, we have the problem that transfer and synthesis may not be identical.

<Chard narrates over diagram showing cross-section of a chorionic villus>

This is a diagram of cross-section of a chorionic villus. Surrounding it is the maternal intervillous space. There are areas of trophoblast, the so-called thin syncytiotrophoblast, overlying foetal[1] capillaries which are obviously specialised for the transfer functions. There are other areas, much thicker trophoblast, which are specialised for the synthetic functions. Notice that the two areas are not the same. If we measure synthesis, we may not necessarily measure transfer. The only indication that we have that the two are related is the fact that placental function tests give us useful clinical information.

The second major area of problem is that of the overlap between normal levels and abnormal levels.

<Chard to camera>

In other words, we cannot say definitively that a given HPL level says that the child is normal or abnormal. Within the normal range, there are bound to occur some cases which subsequently turn out to have had something wrong with the pregnancy. Within the range of HPL or oestrogen levels, which apparently are low or abnormal, we are going to get some normal pregnancies. For any of these tests, there will be a substantial incidence of both false positives and false negatives. And there is no current reason to suppose that this problem can be overcome. The point it does make, and this is very key, is that the results of such tests must always be treated in

[1] Spelt 'fetal' on diagram in film. For consistency the spelling 'foetal' has been used in this transcription.

terms of risk, never as a guarantee that a pregnancy is normal or abnormal. A situation rather similar to the finding of a high blood pressure where, for example, if we find a patient with a diastolic blood pressure of 100, we know that that pregnancy is at greater risk than if the blood pressure were normal. But it doesn't guarantee that risk. Most children of pregnancies of a diastolic blood pressure of 100 are, in fact, normal. Many pregnancies associated with low HPL or oestrogen levels are, in fact, normal. All that it is telling us is that the risk is increased.

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<Chard to camera and then over graph showing hormone level variation during gestation period>

Now, one of the principal reasons for this overlap between normal and abnormal is the variation which exists in the measurement of the normal range or, for that matter, of the abnormal range. And this variation is made up of two parts: there is a biological variation and added to that is what I have chosen to call an assay variation, but that must also include variation in the collection of the sample. Obviously, the bigger the assay variation, the wider will be the range and the less sensitive will be the test. This added variation is one reason why urinary oestrogens are not as popular as they used to be. The problems of accurate sample collection over a 24 hour period are such as in many hands to add immensely to the normal range and therefore to the sensitivity with which it is possible to detect abnormality.

<Chard to camera>

Now finally, while talking about generalisations and problems, a brief word about serial samples. The supposed reason for doing serial samples is that it will show us when a test or when a pregnancy is changing with respect to time; in other words that the levels will fall and indicate a high level of risk. Now this, indeed, happens that serial samples will show you a fall, but that in reality is rare and often associated with complications such as placental abruption about which there is little we can do. Much more important as a reason for doing serial samples is the fact that the more

samples we have from a given patient, the more confident we are of where that patient lies in relationship to the normal range. A series of samples, just below the lower limits of normal, are of more serious import than a single sample which is substantially below those normal limits and that is the value of serial samples.

<Chard narrates over diagram showing pathways of hormone production associated with the foetoplacental unit>

Now, to turn now to the two principal specific examples of placental function tests: first, oestrogens. The reason why, in principle, the measurement of oestrogens is more valuable than that of any other test is that they are synthesised not just by the placenta but by the foetus as well. Because they depend for their production on the production by the foetal adrenal and the foetal liver of steroid precursors, which then are converted to oestrogens by the placenta and therefore when we measure oestrogens and in particular oestriol, we are determining not just that placental conversion but also the supply of the precursors from the foetal adrenal and from the foetal liver. Therefore, in principle, if we have a pregnancy in which the foetus is specifically at risk, in the presence of a normal placenta, then oestrogens will reflect that situation. Obviously, placental specific tests, such as placental lactogen, will not reflect that situation. In reality, of course, such situations are rare. Most of the things which go wrong in late pregnancy are based on what one can loosely and rather badly term placental insufficiency. This is the reason why in real life, measurements of placental function, such as HPL, and measurements of foetoplacental functions, such as oestrogens, can be remarkably similar.

<Chard over diagram showing levels of urinary oestrogen during gestation>

This is a simplistic, in many ways, picture of the use of urinary oestrogens, still the classic and most widely used test or biochemical test of foetal wellbeing in late pregnancy, and shows with the solid lines, here and there, the normal range for urinary oestrogens, and in the shaded area down here, the area in which a pregnancy would be defined as being at risk. This is the broadest and simplest interpretation but remembering, as we said before, that within the normal range there

will be abnormals and that within even the grossly abnormal range, below the absolute lower limits of normal, there will, nevertheless, on statistical grounds be some normal cases.

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<Chard to camera and then over diagram showing levels of human placental lactogen during gestation period. Interspersed with talk to camera>

Now, I would like to turn briefly to human placental lactogen, a specific product of the human placenta, somewhat analogous chemically to pituitary growth hormone and prolactin. Again, the interpretation of placental lactogen levels superficially is simple. We prepare a normal range, shown here again by these solid lines. In relationship to this normal range, we define an area usually quantitating at somewhere below 4 milligrams per litre for the last few weeks of pregnancy, in which if the levels fall in this area, we determine that that pregnancy is at risk. And this would be almost, but not completely, regardless of the presence or absence of a specific complication, such as pre-eclampsia. As I mentioned before, obviously the more levels that we have which fall within a given range, the more confident we are that that patient genuinely bears that relationship to the normal range.

Now, there is a problem which arises, particularly in the case of rhesus isoimmunisation and of diabetes, which is that the known overgrowth of the placenta in those conditions leads to actually high levels of HPL. This is shown here.

<Chard narrates over chart comparing HPL levels in uncomplicated and complicated diabetics during gestation period>

If we take diabetic pregnancies which are uncomplicated, which means that the outcome for the foetus is satisfactory, we get this pattern: here, we have our normal range as before with the mean and 2 standard deviations on either side of it, and here we have the mean for, in quotes, normal diabetic pregnancies with its variation around it. Notice that this is higher than the normal range. If we now take the

complicated diabetic pregnancies, we find this picture. Now the levels are lower than the normal range but, of course, for this test to be useful, these levels must not be compared with the normal range for totally normal pregnancies. In a diabetic pregnancy, we have to take account that the normal range is, in fact, a higher than normal range. If we said that we were going to use for most pregnancies a cut-off point at 4, for diabetics it is more appropriate to take a cut-off point at 5 milligrams per litre, which then hopefully should include the majority if not all, as always, of diabetic pregnancies in which complications subsequently arise.

<Chard narrates over chart showing HPL levels as indication of foetal risk during gestation period, interspersed with talk to camera>

One of the things which we and others have been closely involved in for several years is the concept of using this type of test as a general screening test of foetal risk, not just in pregnancies defined as being at high risk for some other reason but in all pregnancies. And this picture shows the results of one such study from St Bartholomew's on this question, where we took a very, very large group of pregnancies in sequence and excluded from those cases in which there was some obvious clinical abnormality. In every case, we measured HPL levels at 36 weeks and quite often had serial levels on all these subjects. We then analysed the incident of foetal problems in these cases. For levels in the upper end of the normal range, the overall risk to the foetus, such as growth retardation, foetal stress, intrauterine death, was 8%. In the lower end of the normal range, it was 13%, and below the normal range rose to 30%.

Remember that these, at least when first seen, were clinically normal cases. What we see here is a biochemical test making a primary diagnosis of foetal risk. In conclusion, I would like to suggest four developments which are likely for the future. First, there will be new ways of doing old tests. There will hopefully be ways in which we can measure HPL and oestrogens, even in an obstetric side room. There will be new materials. I mentioned at the beginning the new placental proteins, SP1, PP5. Preliminary indications are that SP1 may be a better monitor of foetal wellbeing than HPL.

<Chard narrates over diagram illustrating measurement of placental transfer using selenomethionine>

There will be new concepts, such as measurement of placental transfer. Attempts have been made in this direction by measuring the uptake of a radioactive amino acid, selenomethionine by the uterus and its contents. So far and obviously with the associated risks, this type of technique has not received widespread acceptance, but it illustrates a concept, which is my belief should go substantially beyond the rather course tests of synthetic function which are available to us at the moment.

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<Chard to camera>

Finally, I would like to suggest that we may well finish up in the situation where tests of this type are not just regarded as something that we should do on an inpatient at particularly high risk but something that we should consider doing on all patients, regardless of whether they appear to be at clinical risk or not, because we know that there are a proportion of abnormal pregnancies which we only know about when the child is delivered. Some of those may be picked up by a routine biochemical test. Which biochemical test, at this moment, one would probably say it will be either oestrogens or HPL or, perhaps, both. Screening has been shown using both techniques. Maybe for the future, it will be one of the newer placental proteins as well or instead of. The concept of screening, the concept of using them broadly in all pregnancies is one which I am certain must arrive. Thank you.

<Chamberlain and Chard seated for discussion to camera>

<Chamberlain>

Thank you very much, Professor Chard. That's a very clear account of the present position and a glimpse into the future of what may come. May I ask you first of all

about the last point you raised, the screening function of these tests? Do you see that in the problems we have now – money and lack of resources – do you see any screening tests being used either singly or in groups to possibly replace what many of us feel are the less useful parts of antenatal care, the mid-trimester laying on of hands? Do you think it could be done in the practical circumstances of laboratory / clinical load now or in the immediate future?

<Chard>

I believe it could be done. Certainly, it is technically feasible. One might like to think of this in terms of cost benefit. We now have a routine practice at St Bartholomew's where HPL levels, but it could equally be oestrogen levels – we happen to have selected HPL, are measured at the 32nd and the 36th week. The cost of each test, and this includes all overhead, labour, reagents and sample collection, is roughly £2. We are therefore suggesting the addition of about £4 to the other costs associated with antenatal care. To demonstrate that that is truly cost effective is, of course, difficult because we have the difficulty of ascertaining have we really pre-empted a situation which would have led to subsequent economic loss. That's a difficult subject, nevertheless, I firmly believe that it would be a logical addition to antenatal care because this type of test has been proven in appropriate studies to be equal in its implications to many of the other things which we do. It would probably be equal as a parameter of risk overall to the measurement of blood pressure.

<Chamberlain>

Do you think it could replace anything and thus become even more cost effective?

<Chard>

I would not like to think that it replaced any of the standard clinical measures applied as part of normal antenatal care.

<Chamberlain>

Even though we could show, and as you well know, others have shown this, that many of the standard measures, as we call them, are grossly ineffective as a pick-up of high risk foetuses. Remember Underhill and Beazley's work about uterine size done clinically, how poor that was as a predictor. Possibly some of these clinical measures we should be looking at and if we could show them to be ineffective that possibly the biochemical tests might be better than them.

<Chard>

Unfortunately, it would be reasonable if we said that we lay hands on the abdomen in order to assess size and growth of the child; a good case could be probably made out for not examining the pregnant abdomen. However, there are other things we are looking for in an abdominal examination, such as the lie and position of the foetus which, unfortunately, are irreplaceable at this time.

<Chamberlain>

Oh, I quite agree, that's later on. I was thinking in the mid trimester of pregnancy. I quite agree, past 32 weeks, you're looking for presentation and lie and things, but in the mid-trimester pregnancy, it's possible that it might be replaced, shall we say, by ultrasound measurements being done?

<Chard>

It should, certainly in the mid trimester, there's every reason to think that ultrasound would be a perfectly reasonable replacement for manual examination of the abdomen in mid trimester.

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<Chamberlain>

May I come back to possibly something of your experience in the new field, the pregnancy beta-1-glycoproteins? Can you tell us a little bit about your experience of these in use in pregnant women?

<Chard>

Yes. First, I should perhaps say a word about nomenclature. This is, or appeared, on the first graphic as SP1, which stands for a very long German name. The English version of this name is pregnancy specific beta-1-glycoprotein. It is a large molecular weight protein, apparently specific to the human placenta and to one or two trophoblastic tumours. Its measurement is very easy, much easier, for example, than HPL or oestrogens because it circulates at extremely high levels. Our own preliminary experience and that of other groups has been that as a test, for example, of intrauterine growth retardation, it is actually rather superior both to HPL, which on the face of it comes from exactly the same site, and to oestrogens which come at least partly from the same site. In one series, which we published, our pick-up rate of growth retardation was better than 70%. However, one must always be a little bit cautious about preliminary findings. All tests, when they have first been introduced, have been associated with exceptionally good figures which show them to be superior to all existing tests. All too often with experience, it is found that it is very similar to an existing test and I think we will probably have to wait another year or two before we determine whether SP1 would, for example, be a valid replacement for HPL. It may be, but I think we need a little bit more experimental evidence.

<Chamberlain>

The tests you tell us of obviously are all developing, going into routine work and some of them are going out of the other side obviously. For instance, it was on your list, but you put it a bit to one side, progesterone, which is one of the first tests that used to be used, pregnanediol in the urine and progesterones as a measure of foetal wellbeing. Where does that stand now in your opinion?

<Chard>

In my opinion, and it has to be my opinion because there is no current evidence, the measurement of blood progesterone would be of quite equivalent significance to HPL or SP1 or any other specific placental product; unfortunately, of course, no one has really addressed blood progesterone in this context, not in the full sense of a clinical trial. Now, the earlier work, of course, with urinary pregnanediol suffered from some of the disadvantages which I pointed out with respect to urinary oestrogens – depended upon 24 hour urine collections. It depended upon measurement at a time when some of the concepts of quality control within the laboratories had not been introduced. It depended upon the measurement of a material where you were not only determining how much was being produced you were also determining how the mother metabolised it. I think this combination is the reason why pregnanediol in urine never became popular. I think progesterone in blood would be found to be very similar to HPL or another specific placental product. I can see no obvious theoretical reason, therefore, why we should necessarily re-explore it except purely at a research level.

<Chamberlain>

Yes, it does correlate well with the bulk of trophoblastic tissue. Does it correlate well, plasma progesterone levels with foetal outcomes, which is what we're really measuring?

<Chard>

All of them correlate with the bulk of trophoblastic tissue. This is almost by definition since the trophoblastic tissue is the origin. I can't answer how in a specific clinical setting, it would correlate with foetal outcome. However, if we agree that it is likely to be identical to HPL, then it would correlate with foetal outcome to the extent, which is what we're doing with so many of these tests, that by measuring the function of the placenta, we are indirectly measuring the function of the foetus.

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<Chamberlain>

Another set of tests which were thought hopeful at one stage, that went out, were the transaminases on the grounds that they relate in other parts of the body to hypoxia, heart disease, for instance, but they were shown to have little use, I believe, in the foetus. Would you like to comment on that for a moment?

<Chard>

Yes, there were really two problems here. In this context, one would be looking for the enzyme as a measure of tissue damage; in other words, damaged cells release that enzyme and thereby cause elevated blood levels, exactly in the same way as a myocardial infarct. Now first, placental damage of that acute sense is probably fairly rare. The vast majority of things which go wrong in a pregnancy are really a rather chronic process. Therefore, cases where you could specifically say there had been a big release were probably few and far between and anyway were clinically very obvious indeed, such as a placental abruption. The second problem with enzymes, enzymes of any type, was of course that their measurement can never be quite as precise as that, for example, of some of the hormones or other proteins. As a result, you're always going to get an unacceptably broad normal range, and if you have a broad normal range it becomes very difficult to accurately distinguish abnormality from that range. It's a measurement problem and I think a conceptual problem as well.

<Chamberlain>

That really leads me to my last question which is along the earlier part of your talk. The problem of setting your normals on a range seems to be a mathematical thing; do you believe that one should set it on a 1st or 2nd standard deviation, on a set of percentiles, how do you go about this? For instance, for HPLs you've been so involved with the basic work on, how do you set your normal range on that?

<Chard>

I now believe that the correct way for a unit entering this for the first time and wishing to generate their own data as opposed to accepting published data, which can be dangerous, would be to take, and I would suggest this could be done at random regardless of normality or abnormality, somewhere between 1 and 400 cases in the last few weeks of pregnancy, for example, at 36 weeks, to measure using their method, hopefully a well-established method, the levels of HPL, it might be, in those cases; then to analyse them to ascertain what is the 10th centile of the whole range they have obtained – by 10th centile I mean that level below which 10% of the levels lie; to declare that 10th centile as being the lower end of their normal range. It's much better now to use centiles rather than standard deviations for the reason that there is not a normal distribution in any of the normal ranges of the placental products.

<Chamberlain>

And therefore on that 10th centile or below the 10th centile would be the group that was at higher risk and thus one would pay more attention to them, I imagine?

<Chard>

That would be one's best definition of a potentially high-risk group.

<Chamberlain>

Thank you very much. Thank you for taking us through the problems and putting them so clearly to us and thank you for showing us some idea of what could come in the future. We're grateful to you and that's the end of this programme.

<End credits>