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Tests of foetal lung maturity: a review

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Introduction

Prematurity and Respiratory Distress Syndrome (RDS) are major causes of neonatal morbidity and mortality [1,2]. The incidence of prematurity is high world-wide, but the incidence of RDS, which occurs only in premature babies, varies in different ethnic groups which have comparable rates of prematurity [3,4]. The disparity between the incidence of RDS in relation to prematurity in different ethnic groups has not been satisfactorily explained. One of the difficulties in establishing firm evidence of discordance between the incidence of RDS and gestational immaturity is the difficulty of establishing the correct gestational age of a foetus in the absence of accurate menstrual dates. The gestational age assessed from the date of the last menstrual period is not always a true estimate of the age of the foetus because of aberrant menstrual episodes around conception even in women who keep accurate records of their menstrual bleeds. Inaccurate recall of menstrual episodes in women who do not keep records of their menstrual periods makes estimation of foetal age from the last menstrual period impossible. Foetal lung maturity does not always synchronize with foetal gestational maturity because of metabolic or obstetric factors such as diabetes mellitus, toxaemia of pregnancy and placental insufficiency, operative primarily in the mother, which affect foetal lung maturity [5–7]. There has therefore been a need for objective assessment of foetal age and foetal lung maturity whenever active intervention in delivery or delay of delivery of an infant is to be practised. This need has been met by various diagnostic procedures using different indices of foetal maturity. This review attempts to highlight some of these procedures in so far as they have achieved the objective of providing reliable indices of foetal gestational or lung maturity. For ease of discussion, we have classified the currently used diagnostic indices of foetal maturity into those dependent on assessment of (1) intra-uterine foetal size, (2) indices of skeletal maturity, (3) soft tissue imaging characteristics, and (4) physical or biochemical characteristics of amniotic fluid.

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Intra-uterine foetal size

Measuring the height of uterine fundus with a tape measure applied to the anterior abdominal wall from the top of symphysis pubis, and measuring to the nearest 0.5 cm, Quaranta *et al.* [8] showed that mean fundal height in normal singleton pregnancies increases from 19.8 cm at 20 weeks to 36 cm at 39 weeks gestation. From the mean fundal height measurements, a nomogram showing 10th, 50th and 90th percentile curves of symphysial-fundal height in relation to gestational ages was also drawn, thereby establishing a 'reference range' for fundal height from the 20th week of gestation to term. Uterine content — foetus, placenta, and amniotic fluid — together with uterine musculature contribute to the size of uterus, which correlates with the gestational age of the foetus in normal pregnancies. Any departure from the normal, which affects any of these variables, may render the estimation of foetal gestational age by this method unreliable. Multiple pregnancies, co-existing uterine masses, and hydramnios are some of the common causes of discrepancy between fundal height and gestational age. There is, as with all measurements, an unpredictable observer variation in the physical measurement of fundal height which may reduce further the usefulness of this clinical assessment of gestational age. Perhaps the greatest advantage of this method of assessment

is its non-invasive nature which carries no hazard for the mother or the foetus. The only requirement is the skill in clinical examination of the obstetrician and a tape measure. This type of assessment, however, does not give any information on foetal lung maturity.

Indices of skeletal maturity

Foetal head circumference increases with gestational age. The biparietal diameter (BPD) of the foetal head, which can be measured by ultrasound [9], has been used in the assessment of the head circumference. Ultrasonographic measurement of BPD reliably estimates foetal gestational age from 20 weeks onwards. A BPD of ≥92 mm is known to correlate with foetal gestational age above 37 weeks and foetal lung maturity [10–12]. However, the predictive value of this measurement alone for mature lungs is poorer than using the lecithin/sphingomyelin (L/S) ratio. Only 33% of mature foetal lungs predicted by the L/S ratio was correctly predicted by ultrasound measurement of BPD [12]. Below 37 weeks gestational age (menstrual age), however, ultrasonographic measurement is notoriously unreliable for predicting foetal lung maturity. There is a false-positive prediction of lung maturity of greater than 80% below 37 weeks gestation. This improves to less than 10% false-positive prediction when the menstrual age of the foetus is greater than 37 weeks [11]. This puts a considerable limitation on the use of ultrasonic measurements for determining lung maturity in pregnancies younger than 37 weeks.

The appearance of epiphyseal ossification centres demonstrated by sonography or radiography has been shown to be useful in predicting foetal lung maturity [13]. Mahony *et al.* [14] demonstrated perfect correlation between visible proximal humerus epiphysis and mature amniotic fluid profile, while the proximal humerus epiphysis was invisible in foetuses with immature amniotic fluid lung profiles.

Radiological measurement of foetal heads, or demonstration of the appearance of ossification centres, which can be equally achieved with ultrasound scanning, has been dropped where ultrasound facilities are available. The hazard of radiation cannot be justified in the presence of ultrasound scanning which, as of now, has not been shown to present any hazard to the foetus.

Soft tissue imaging

Both the foetal lung and the placenta have been studied for ultrasound characteristics which may correlate with foetal lung maturity [11,15]. Again, as with the measurement of BPD of the foetal head, there is a better correlation between soft tissue imaging characteristics and menstrual age of the foetus than with foetal lung maturity [11,16]. Mullin *et al.* [12] have demonstrated that free-floating particles (liposomes) in amniotic fluid, visible under ultrasound scanning, correctly predicted 35% of mature L/S ratio in non-diabetic pregnancies. When the presence of free-floating particles in amniotic fluid is combined with BPD of >92 mm, the predictive value of ultrasound scanning is enhanced [12]. Ultrasound soft tissue imaging is more frequently used to locate the placenta prior to amniocentesis.

The non-invasive nature of ultrasonography is the major attraction of this method for determining gestational or lung maturity. The high personnel and equipment costs entailed make this method unavailable to many obstetricians in the developing world.

Physical and biochemical characteristics of amniotic fluid

Amniotic fluid, a product of chorionic tissue, and the fluid in which the foetus lies and releases products of its metabolism, is a natural target for the study of foetal maturity. Various physical and biochemical characteristics of amniotic fluid have been and are being used to study different aspects of foetal physiology. Gestational maturity and foetal lung maturity have been studied extensively by assessment of changes in amniotic fluid physical characteristics caused by the presence of cellular and lipid particles released into the fluid from foetal tissues [17–21]. The physical phenomena that have been used to predict foetal lung or gestational maturity include amniotic fluid microviscosity measured by fluorescence polarization [17,18], spectrophotometric absorbance of amniotic fluid at 650 nm [19,20],

and electrophoretic mobility of amniotic fluid liposomes [21]. The physical phenomena measured are mainly affected by the lipid content of amniotic fluid. As the lipid content of amniotic fluid is not exclusively of surfactant origin, the measurements could sometimes be inaccurate indices of foetal gestational or lung maturity.

Avery and Mead [22] established that lung atelectasis and respiratory distress result from poor formation and maintenance of phospholipid-rich surface-active alveolar lining. Following this work, considerable attention has been given to the study of lung surfactant and its various subfractions released from foetal lungs into amniotic fluid. Surfactant is a lipoprotein complex secreted by type II pneumocytes of the lung alveoli [23]. The phospholipids lecithin and sphingomyelin, were shown by Gluck *et al.* [7] to constitute the largest amount of the surface-active material of surfactant. The establishment of the L/S ratio as an index of foetal lung maturity was the result of this work. The method of determination of L/S ratio described by Gluck *et al.* [7] is thin-layer chromatography (TLC) of lipid solvent extract of amniotic fluid phospholipids. The method is labour intensive and prone to analytical imprecision because of the multiple steps in the analysis which may result in unpredictable losses of the extracted phospholipids. Variations on the method of Gluck *et al.* [7] have been developed to improve the analytical performance and predictive value of the assay [24–31]. An L/S ratio of 2 or greater is generally accepted as correlating with mature foetal lung [7]. The predictive value of L/S ratio of 2 or greater for mature foetal lung is 98% while the predictive value of a ratio of less than 2 for immature foetal lung is only 37% [32]. This is a reflection of the poor specificity of L/S ratio as an index of foetal lung immaturity. Only limited attention has been given to measurement of the concentrations of lecithin, sphingomyelin, and other phospholipids in amniotic fluid. The analytical difficulty of quantitating these phospholipids has been a major constraint to using the concentrations of the phospholipids of amniotic fluid as indices of foetal lung maturity. Tedious methods for the estimation of lecithin in amniotic fluid have been described [33,34]. An enzymic method for estimating amniotic fluid lecithin and sphingomyelin has recently been established [35]. Using this new method, Ogunkeye and Otubu [36], found a wide range of total lecithin and sphingomyelin in amniotic fluid of infants who had no respiratory distress syndrome after delivery. The fact that many infants with an L/S ratio of less than 2 fail to manifest RDS suggests that the relative concentrations of lecithin and sphingomyelin is probably not the most important index of lung maturity even if only the lipid components of surfactant are considered. Larger studies of total concentrations of lecithin and sphingomyelin should show the importance of the absolute concentrations of the major phospholipids of surfactant in attainment of lung maturity. The value of the use of amniotic fluid phospholipids to predict foetal lung maturity has been improved by the recognition of the importance of the appearance of phosphatidylglycerol in amniotic fluid which signals foetal lung maturation. Phosphatidylglycerol is secreted from alveolar cells very late in pregnancy, and is also released into amniotic fluid like other lung phospholipids [37]. Hallman *et al.* [38] have shown that the presence of phosphatidylglycerol in amniotic fluid more consistently correlates with foetal lung maturity whatever the L/S ratio. The very low concentration of phosphatidylglycerol in amniotic fluid (as low as 12 μmol/l) presents some analytical difficulties in detection and quantitation [39]. Analytical methods for detection or quantitation of phosphatidylglycerol in amniotic fluid include two-dimensional thin-layer chromatography [25], enzymic colorimetric assay [40], and immuno-agglutination assay [41]. The chromatographic methods of amniotic fluid phospholipid determination are labour intensive, taking 3 h for a skilled technologist. Such time delay may be detrimental to good obstetric practice. There has therefore been much interest in the development of simpler tests on amniotic fluid which indicate foetal lung maturity or immaturity. Some of these tests exploit the detergent activity of surfactant on the formation and stability of air bubbles in liquids [32,42,43]. Although these tests are simple and can be carried out in a short time by non-laboratory trained personnel, the predictive value of the tests is poorer than phospholipid profile assays. Other alternative tests to L/S ratio determination are aimed at developing less labour-intensive but more precise assays of

the various amniotic fluid phospholipids [35,44,45]. Less attention has been devoted to the study or the estimation of apoproteins of lung surfactant which may be no less important determinants of lung maturity than the phospholipids. In-vitro studies have shown that surfactant proteins enhance the rate of surface-film formation in alveoli [46–48]. The better efficacy of natural surfactants, which contain about 1% of lower molecular weight proteins, than synthetic protein-free mixtures of phospholipids in preventing RDS in immature lambs [49], reinforces the importance of surfactant apoproteins. The development of tests for the quantitation of surfactant apoproteins [50,51] is therefore a step in the right direction.

Conclusions

Steps in the right direction have certainly been taken. The recognition of the role of surfactant phospholipids and apoproteins in alveolar function has led to the successful use of artificial surfactants in the treatment of RDS [52,53]. Many of the tests that have been discussed in this review have been, and are being used in spite of the limitations of the tests, for decision making in obstetric practice. However, the real index of foetal lung maturity remains elusive. The relative rarity of RDS in Negroid infants compared to Caucasian infants of similar gestational age and birth weight is well documented but unexplained. This may be one of the keys to the complete understanding of the Respiratory Distress Syndrome.

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