

## SIMPLE TEST OF FOETAL MATURITY

by

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### *Introduction*

Much has been written on amniotic fluid and many laboratory methods for estimating foetal maturity but as yet practically nothing is written on visual assessment of amniotic fluid for predicting foetal pulmonary surfactant maturity. In the course of the study of foetal lung maturity in high-risk pregnancies (unpublished data), we were struck by the observation that obvious presence of vernix particles in the amniotic fluid and/or a distinct turbidity correlates very well with the foetal maturity and in these foetuses hyaline membrane disease is unlikely to develop. The present study was undertaken to confirm and enlarge upon these findings and compare the results of visual examination of amniotic fluid with Lecithin/Sphingomyelin (L/S) ratio or occurrence of hyaline membrane disease (HMD) in the neonate.

### *Material and Methods*

Group I: Seventy-one patients (12 preterm and 59 term pregnancies) with a definite indication of amniocentesis were selected. The following categories of patients were studied; (a) patients re-

quiring elective caesarean section near term, (b) patients with suspected intra-uterine growth retardation, (c) patients with a complication of pregnancy i.e. hypertension, renal disease, pre-eclamptic toxæmia (PET), gestational diabetes etc. requiring termination, and (d) patients with mistaken dates.

Amniotic fluid specimens were studied visually by holding the fluid in a clean test tube against light for the presence of vernix caseosa particles and/or turbidity. The same fluid was subjected to the estimation of L/S ratio. A total of 69 patients who delivered within 72 hours of amniocentesis were examined for the presence or absence of HMD in the neonate.

Group II: In 10 cases, where emergency caesarean section was performed between 32 to 36 weeks of pregnancy for various obstetrical indications. In these cases visual examination of amniotic fluid was compared with the occurrence of HMD in the neonate. Estimation of L/S ratio was not done in these cases.

The infant was diagnosed to have HMD, if 5 of the following 6 clinical criteria were satisfied; preterm infant (less than 37 weeks), respiratory rate of 60/min. or above within 4 hours of birth, intercostal recession and/or substernal retractions, grunting, cyanosis in the room air, and a normal skiagram of the chest or compatible with HMD. Transitional tachypnoea

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was diagnosed when the respiratory rate exceeded 60/min. beyond the first 4 hours of life with no grunt or recession.

*L/S ratio estimation:* The extraction of lipids from the amniotic fluid was undertaken within 12 hours of collection according to the method of Folch *et al* (1957). The phospholipids were separated by thin layer chromatography using the solvent system described by Rouser *et al* (1970) and the phosphorus content of Lecithin and Sphingomyelin spots was determined by the method of Barlett *et al* (1959) and the ratio was calculated.

L/S ratio of 1 was taken as immature, between 1 and 2 as intermediate and 2 or above as mature.

### Results

*Comparison of visual assessment of amniotic fluid and L/S ratio for foetal lung maturity (Table I):* Fifty-one am-

ed to be turbid, of which 15 did not contain any obvious vernix flakes but showed presence of free fat globules in the unstained smear. All of these cases had a L/S ratio of 3 and above.

Five specimens were assessed as clear or 'off clear'. Two of these had a L/S ratio of less than 2. Delivery was delayed in both these cases until the foetus was mature. In both the cases the indication of amniocentesis was mistaken dates. The other 3 had a L/S ratio of more than 2. All the 3 cases were of PET and amniocentesis was performed at 35 to 36 weeks of gestation.

*Relationship between the visual assessment of amniotic fluid and the development of HMD (Table II):* None of the neonate with amniotic fluid positive for vernix flakes and/or turbidity in group I and II developed HMD. Only 1 child developed transient tachypnoea.

TABLE I  
Comparison of Visual Examination of Amniotic Fluid and L/S Ratio

Visual examination of amniotic fluid	No. of cases	L/S ratio			
		Less than 1.5	1.5 to 2	2 to 3	More than 3
Turbid with flakes	12	0	0	0	12
Turbid without flakes	15	0	0	0	15
Clear with flakes	39	0	0	5	34
Clear without flakes	5	0	2*	1	2
Total	71	0	2	6	63

\*Delivery was delayed in these two cases.

niotic fluid specimens in group I were noted to have obvious vernix flakes. All had a L/S ratio of 2 and above. Vernix flakes when stained with Nile blue sulphate, appeared as large clumps of cells scattered here and there in an orange stained lipid material.

Twenty-seven amniotic fluids were not-

In group II cases, 2 out of 7 with absent vernix flakes developed HMD and 1 neonate developed transient tachypnoea. The diagnosis was confirmed at autopsy. Two cases of group I with absent vernix flakes and L/S ratio of less than 2, the delivery was delayed till the foetus was mature.

TABLE II  
Relationship Between Visual Examination of Amniotic Fluid and HMD

	Visual examination of amniotic fluid	No. of cases delivered within 72 hours	No. of HMD
Group I	Turbid with flakes	12	0
	Turbid without flakes	15	0
	Clear with flakes	39	0
	Clear without flakes	3	0
Group II	Clear with flakes	3	0*
	Clear without flakes	7	2

The child developed transient tachypnoea.

### Discussion

Vernix caseosa is a mixture of sebum, desquamated cells and lanugo. As the pregnancy progresses and sebaceous gland function increases and more fatty detritus is produced. This material when added to exfoliated skin squames forms vernix caseosa. It is suggested that orange cells (fat cells) are probably derived from the exfoliated vernix caseosa and/or foetal sebaceous glands and can be detected in the amniotic fluid as large flakes detectable with naked eye and microscopic clumps lying free in the amniotic fluid. This is in accordance with the observation that vernix caseosa on the foetal skin tends to diminish in mature baby.

The value of turbidity of liquor amnii in assessing foetal maturity was established by Woyton (1963). He correlated the fat content and turbidity of liquor amnii with foetal maturity. He observed that there was rise in fat content and turbidity of amniotic fluid as the pregnancy advanced. This was attributed to the maturity of epidermis, especially the stratum corneum. Our observation of microscopic evidence of free fat not associated with cells indicate its origin from

active sebaceous glands of the foetal skin. Thus vernix particles and/or turbid amniotic fluid are crude quantitative measure of fat content of amniotic fluid and presence of either or both of these must be taken into account in assessing the foetal maturity.

It would appear from our results that with obvious vernix particles and/or turbidity of the amniotic fluid, one can predict the foetal maturity as well as the development of HMD. However, in absence of vernix caseosa flakes and/or turbidity of amniotic fluid one has to estimate L/S ratio or other parameter to assess the foetal maturity. 3 cases of PET with clear liquor obtained at 35 to 36 weeks gestation, had L/S ratio of more than 2. The assessed age at birth in all the three cases was 36 weeks and none of them developed HMD. Presence of PET can explain acceleration of foetal lung surfactant maturity in these cases.

Of 7 preterm infants in Group II with clear amniotic fluid and delivered by caesarean section, 2 developed classical HMD. This suggests that preterm infant with clear amniotic fluid born by abdominal route has 28 per cent chance of developing HMD. It is therefore suggested that visual assessment of amniotic fluid

should be done in all cases during delivery so that 'at risk' neonates with clear amniotic fluid can be transferred to special care units where early therapeutic measures can be taken to minimise the damage by respiratory distress syndrome. On the other hand, amniotic fluid showing flakes of vernix caseosa and/or turbidity virtually excludes the risk of HMD and other conditions such as intrauterine pneumonia, early onset of group B streptococcal infection and congenital malformations should be excluded by appropriate laboratory studies.

Thus the visual examination of amniotic fluid attains greater significance when tests for foetal maturity are essential eg. high-risk pregnancies but in whom the amniotic fluid is contaminated by blood and/or meconium making other tests unreliable. No logical explanation can be given for the correlation of foetal lung maturity with the presence of vernix caseosa flakes and/or turbidity of the amniotic fluid.

#### *Summary*

The visual assessment of amniotic fluid was compared with Lecithin/Sphingomyelin (L/S) ratio as a measure of foetal lung maturity in 71 cases requiring amniocentesis. All patients with obvious vernix flakes and/or turbidity of amniotic fluid had mature L/S ratio, whereas those with clear liquor and negative for

vernix flakes had L/S ratio ranging from immature to mature. A total of 79 patients who delivered within 72 hours of visual assessment of liquor amnii were assessed for the presence of Hyalin membrane disease (HMD) in the neonate. No case of HMD was associated with a positive vernix caseosa flakes and/or turbidity of amniotic fluid. However, 2 out of 7 foetuses with clear liquor developed HMD. Visual assessment of amniotic fluid thus appears to be a reliable indicator of the development of HMD. This simple observation can serve as a big diagnostic aid in cases where tests for foetal lung maturity are rendered invalid by contamination of amniotic fluid by blood or meconium.

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