AMNIOTIC FLUID SURFACE TENSION – A PREDICTOR OF FOETAL LUNG MATURITY

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SUMMARY

102 samples of amniotic fluid from uncomplicated term pregnancies including those contaminated with blood and meconium were studied for L/S ratio and surface tension (ST) along with the clinical outcome. The mean surface tension of clear amniotic fluid of term pregnancies was 66.9 mN/m (SD-4.18 mN/m). Surface tension (ST) of ≤ 71 mN/m was found to predict L/S ratio correctly in 87.3% of cases and there were 1.26% false positive and 11.3% false negative results. Neonatal outcome was predicted correctly in 83.9% of cases by ST. There was no significant difference (f = .387, p > 0.05) in the ST of clear, meconium and blood stained amniotic fluid. The predictability of ST of blood stained and meconium stained amniotic fluid in relation to clinical outcome was 95.6% and false negatives were 4.34% which were almost similar to that of clear amniotic fluid.

INTRODUCTION

The Lecithin - sphingomyelin ratio (L/S) in amniotic fluid as described by Gluck et al (1971), has become the standard for predicting the risk of respiratory distress syndrome. Though a reliable parameter, L/S ratio estimation requires a complex biochemical analysis by a trained technician, a sophisticated laboratory, a delay of 3-4 days to obtain the results, as well as prohibitive expenditure if it is to be made available on daily basis. In order to circumvent above problems the bio-physical characteristics of amniotic fluid were studied (Clement et al, 1972; Sbarra et al, 1976). However, predictive value of these tests are not

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reliable due to very high false negative rates (Rodrigues et al 1979; Arias et al 1978). Further, in most of the studies amniotic fluid contaminated with blood or meconium were discarded for biochemical and biophysical analysis for predicting lung maturity. But contaminated amniotic fluids are not infrequently encountered during amniocentesis.

The purpose of this study was to evaluate another rapid method for predicting lung maturity by estimating the surface tension of amniotic fluid and also to find out the predictive value of this test when amniotic fluid is contaminated with blood and meconium during the process of sample collection. This method required only 3 minutes of time and no special training was needed for performing the test.

MATERIAL AND METHODS

One hundred and two subjects were enrolled for study from Nehru Hospital attached to Postgraduate Institute of Medical Education and Research (PGIMER) Chandigarh, between December 1988 and January 1990. Patients with pregnancies between 37-42 weeks gestation with no obstetrical, medical and surgical complication and with known last menstrual period were recruited for the study. All selected patients delivered within 72 hrs. of sample collection.

Eight ml of amniotic fluid was collected from the selected cases by methods already described (Dhall et al, 1980).

Samples were then divided into two aliquots, one portion was subjected to estimation of Lecithin and Sphingomyelin by thin layer chromatography as described by Mitnick et al (1980) and other portion was subjected to estimation of surface tension by drop weight method as described below :

PRINCIPLE

The weight of a drop of any liquid is directly proportional to the surface tension of that liquid when passed through a nozzle of critical diameter. Based on this principle Bruni et al (1988) suggested a formula for calculating the surface tension of any liquid :

$$\frac{\textbf{Weight of 50 drops of unknown fluid}}{\text{Weight of 50 drops of distilled water}} = \frac{\textbf{Surface tension of unknown fluid}}{73 \text{ mN/m}}$$

or

$$\text{Surface tension of unknown (in mN/m)} = \frac{73 \times \text{weight of 50 drops of unknown}}{\text{Weight of 50 drops of distilled water}}$$

PROCEDURE

4 ml of fresh amniotic fluid samples were taken for the study.

i. Distilled water was forced by MICROPERFLEX PERISTALTIC PUMP 2132 through a nozzle with an internal diameter of 2 mm.

ii. The flow was adjusted so that distilled water gives 50-65 drops per minute.

iii. 50 drops of distilled water was collected in Laxbro Storage vials and the weight was taken by a digital balance (Mettler DC - 4400).

iv. Three to four ml of amniotic fluid was forced to pass through the nozzle by

peristaltic pump and 50 drops of amniotic fluid was collected in a storage vial.

v. The weight of 50 drops of amniotic fluid was taken by the same balance.

vi. The procedure was repeated twice and the mean weight was taken.

vii. The collection and the weighing procedure was done by nontouch technique by using a forceps to handle the vials.

viii. The surface tension of amniotic fluid was calculated by applying the above mentioned formula.

Neonatal outcome was evaluated by an objective assessment of the condition of the newborn by studying the parameter as described earlier (Rodrigues et al, 1979).

The data were analysed by means of correlation coefficient, analysis of variance and student 't' test wherever appropriate.

RESULTS

A total of 102 amniotic fluid samples were obtained from the selected subjects of which majority (46.2%) were primigravida. Of these 79 amniotic fluid samples were free from any contamination but there were 10 samples which were contaminated with meconium and 13 samples were contaminated with blood.

VALIDITY OF THE METHOD

Before measuring the surface tension of amniotic fluid the validity of the method was confirmed by measuring the surface tension of Ethyl alcohol, methyl alcohol, acetone and 5% Ethyl alcohol in water. It is shown in Table I, that the values of surface tension of different liquid as measured by drop weight method was well compared with the values of surface tension of the same liquids according to the international critical tables (1928).

ANALYSIS OF AMNIOTIC FLUID

To evaluate the method, the surface tension of amniotic fluid of term pregnancies were measured and simultaneously compared with L/S ratio. When values of ST and L/S ratio of clear

Table I

Comparison of surface tension of different liquids according to drop weight method and international critical tables

Suface tension in mN/m according to international critical tablets			Determination by drop weight method	
			ST (mN/m) mean ± SD	No. of determination
1. Methyl alcohol	-	22.61 ± .1	22.50 ± .16	20
2. Ethyl alcohol	-	22.75 ± .3	22.60 ± .19	20
3. Acetone	-	23.70 ± .2	23.82 ± .20	20
4. 5% Ethyl alcohol in water	-	60.7 ± .2	60.55 ± .14	20

amniotic fluid were plotted in scattered diagram (Fig. 1), it was seen that there were significant negative corelation ($r = .315$, $p < 0.005$) between the two

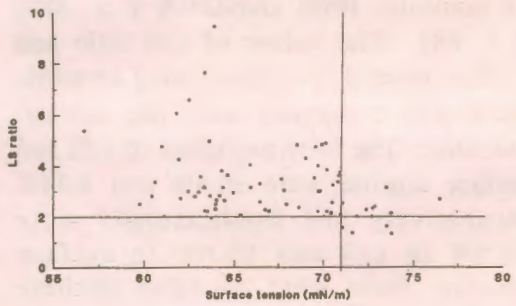

Fig. 1 : Scatter diagram of L/S Vs ST in clear amniotic fluid.

parameters.

The cut off values of ST was calculated to be 71 mN/m (millinewton/metre) (Table II) giving the predictablity of 87.3%, false positive value of 1.26% and false negative value of 11.3%.

The neonatal outcome was studied in all 102 cases. There was no case of respiratory distress syndrome. The false positive, false negative and predictability of surface tension and L/S ratio of clear amniotic fluid were calculated with respect to clinical outcome (Table III). There were no false positives with either of the tests. The false negative with

Table III

Relationship of surface tension and L/S ratio of clear amniotic fluid to pulmonary maturity (N = 79)

	Respiratory distress syndrome (RDS)	No RDS
L/S ratio ≥ 2	0	74
L/S ratio < 2	0	5
ST ≤ 71 mN/m	0	66
ST > 71mN/m	0	13

Table II

Correlation between L/S and ST with different arbitary ST cut off

Arbitary ST cut off	ST	L/S ≥ 2	L/S<2	False +ve	False - ve	Predictability
69mN/m	≤ 69	45	1	1.26%	36.71%	62.02%
	> 69	29	4			
71mN/m	≤ 71	65	1	1.26%	11.39%	87.3%
	> 7	9	4			
73mN/m	≤ 73	71	3	3.79%	3.79%	92.4%
	> 73	3	2			
75mN/m	≤ 75	73	5	6.32%	1.26%	100%
	> 75	1	0			

L/S ratio and surface tension were 6.32% and 16.04% leading to a predictability of 93.67% and 83.95% respectively.

The effect of meconium and blood on amniotic fluid lecithin sphingomyelin ratio estimation is shown in Fig 2, it was found that the mean L/S ratio of amniotic fluid contaminated with meconium (5.67) was higher than that of clear amniotic fluid (3.15) whereas the

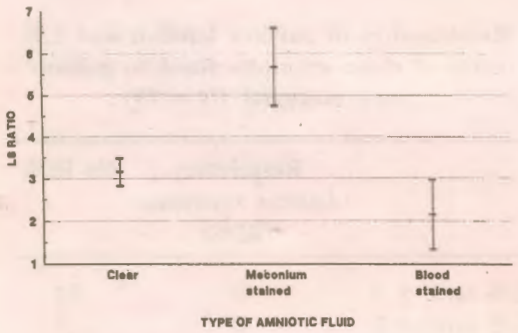

Fig. 2 : Variation of L/S ratio in different types of amniotic fluid.

mean L/S ratio of blood stained amniotic fluid (2.14) was lower than that of clear amniotic fluid. Analysis of variance showed (ANOVA) statistically highly significant difference between the three groups ($f = 17.28$, $p < .001$). But the mean surface tension values of clear, meconium and blood stained amniotic fluid was almost equal (Table IV). There was no significant difference in the surface tension of three different types of amniotic fluid (ANOVA $f = .387$, $p = .68$). The values of L/S ratio and surface tension of contaminated amniotic fluid was compared with the clinical outcome. The false negatives of L/S and surface tension were 30.4% and 4.34% respectively and Predictability were 69.5% in L/S and 95.6% in surface tension, there were no false positive results in either of the tests.

DISCUSSION

The measurement of surface tension of amniotic fluid is a simple, rapid and economical method to predict foetal pulmonary maturity. The basis of this bio-physical characteristics of amniotic fluid is that surface tension will change according to the surfactant content. It will decrease with the increases in total phospholipid concentration of amniotic fluid. The total phospholipid concentration of

Table IV

Distribution of surface tension according to type of amniotic fluid

Type of amniotic fluid	ST ≤ 71	ST > 71	Mean ST (mN/m)	SEM
Clear (79)	66	13	66.99	.47
Meconium stained (10)	9	1	66.33	1.76
Blood Stained (13)	13	0	67.87	.96

Figures in the parenthesis indicate the total number of samples

amniotic fluid was found to be increased by 8 fold from 30 weeks to 40 weeks of gestation (Nelson 1969). Schreyer et al (1974) found that both the total phospholipid level and L/S ratio demonstrated a similar increase in pregnancy. So with increasing period of gestation as the total phospholipid level increases the surface tension of amniotic fluid will decrease. As the surface tension of amniotic fluid indicates the total surface active material (total phospholipid) in the amniotic fluid, it has a theoretical advantage over other method that evaluate only one of the surface active substance in the amniotic fluid. The results of experiment on different liquid of known surface tension confirm that the drop weight method is a reliable index of measuring surface tension.

In the present study the mean surface tension of clear amniotic fluid of term pregnancies was 66.9 mN/m. The highest value was 76.4 mN/m and the lowest 56.6 mN/m. These results are in agreement with those of Enhorning (1964) who in a study of amniotic fluid surface tension of term pregnancies showed that the mean surface tension value was 69.5 dynes/cm and the highest and lowest value were 74.1 dynes/cm and 58.5 dynes/cm respectively. Surface tension of clear amniotic fluid of $\leq$ 71 mN/m was seen to predict a mature L/S in 98.4% cases but surface tension of > 71 mN/m is associated with immature L/S ratio in only 30.7% of cases. A mature surface tension value of $\leq$ 71 mN/m is associated with healthy infants in 100% of cases (negative predictive value).

The L/S ratio is known to be affected by presence of blood or meconium in the sample of amniotic fluid. Gluck et al (1971) noted that packed red cell volume greater than 0.05 ml for 3 ml of amniotic fluid may give false positive results.

In our study it was found that 54% of samples of amniotic fluid contaminated with blood and L/S ratio < 2 i.e. there is a tendency to have an immature L/S ratio in amniotic fluid contaminated with blood. These results are in agreement with the findings of Buhi and Spellacy (1975) that a high amniotic fluid L/S was decreased by the addition of either maternal or foetal serum and the extent of the alteration depends upon the volume of serum added.

It was also found that the mean L/S ratio of meconium stained amniotic fluid was significantly ($t = 4.93$, $p < .001$) higher than that of clear amniotic fluid which indicate that the meconium in the amniotic fluid increases the L/S ratio. This observation is similar to the finding of Kulkarni et al (1972) that presence of meconium in the amniotic fluid made the results unreliable, biased towards a high L/S ratio even when the pregnancy is premature.

When the L/S ratio of blood stained and meconium stained amniotic fluid was correlated with the clinical outcome it was found that there were 30.4% false negative results but no false positive results. The predictability was 69.5%, which was 93.6% with clear amniotic fluid. So L/S ratio estimation to predict lung maturity in contaminated amniotic fluid is not reliable.

In this study, it was fund that there

was no significant variation of surface tension value in presence of blood and meconium in the amniotic fluid. A surface tension value of ≤ 71 mN/m was associated with healthy new born even in presence of contamination. The predictive value of surface tension of clear and contaminated amniotic fluid was almost equal (83.9% vs 95.6%). This observation can be explained by the fact that surface tension of amniotic fluid is the reflection of total surface active material in the amniotic fluid, not related to an individual phospholipid, so whenever there is low concentration of blood and meconium contamination which may occur due to dilutional effect of natural contamination, it will not alter the total phospholipid content and thereby will not affect the surface tension value. But higher concentration of blood and meconium contamination will decrease the surface tension of amniotic fluid. So contamination with higher concentration may give falsely mature surface tension value but never give falsely immature value.

In conclusion - drop weight method of measurement of surface tension is a rapid and bed side method and could be used in a reliable way to determine pulmonary maturity even in presence of contamination and meconium in the amniotic fluid.

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