

Image J Analysis Of Amniotic Fluid Echogenicity And Labor

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Citation

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Abstract

Introduction: The amniotic fluid turbidity increases in late third trimester of pregnancy culminating in Labor at AFOD of around 1.0. The lung skin interactions causes vernix separation and there is increase in size, number and distribution of amniotic fluid particles which contribute to the echogenicity of amniotic fluid. An Objective measurement of amniotic fluid echogenicity is attempted in this study.

Aim: To study the pattern and measure the changes in amniotic fluid echogenicity in late third trimester and correlate with amniotic fluid optical density (AFOD) at labor.

Materials and methods: This observational study comprises 41 singleton pregnancies who underwent transabdominal USG with 3.5 MHz in third trimester till labor. Amniotic fluid echogenicity index is calculated with Image J software by selecting the region of interest and comparison with adjacent structures. Polygonal selection was used for demarcation. The echogenicity index (eAF) at labor was correlated with AFOD at 650 nm. Statistical analysis was done with SPSS 15.

Results: The mean echogenicity index (eAF) at onset of spontaneous labor is 73.1 % SD 8.5 % with significant positive correlation with AFOD. A rapid increase eAF is noted after a value in the range of 60-65 % and within duration of about a week of onset of Labor is taking place at around 70 % (eAF). **Conclusion:** Spontaneous labor appeared to be associated with rise in echogenicity of amniotic fluid.

INTRODUCTION

It is established that the turbidity of amniotic fluid increases gradually and matures at the end of pregnancy. With the advancement in pregnancy in third trimester, the amniotic fluid particles appear and increase in size number and distribution and accounts for the echogenicity visualized in USG. (1,2) It is undoubtedly the vernix separation which appears as amniotic fluid particles and is attributed to the echogenicity in late third trimester (3,4). Many authors have studied free floating particles in amniotic fluid and correlate with fetal maturity (5-7). This phenomenon is also observed in animal studies. (8) In this study an attempt has been made to correlate and quantify the physiological changes in AF with sonological and computer assisted image analysis (Figure 1). A non invasive assessment of amniotic fluid turbidity or optical density has profound implications in prediction, programming and optimizing labor.

The lung skin interactions influenced by several factors causes separation of vernix caseosa. As the lung matures;

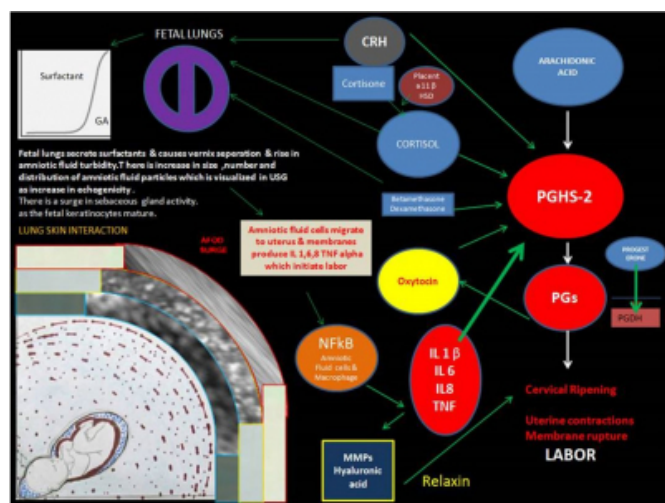
proportionate to the amount of surfactant released more the desquamation of mature fetal keratinocytes takes place (3). The exponential burst of pulmonary surfactant and in turn Amniotic fluid cells and macrophages (9) secrete pro labor cytokines which surge and initiate labor. (10) Labor is in fact an aseptic inflammatory process which involves cervical ripening and accentuation of Braxton Hicks contractions. These events can be interpreted by increasing turbidity of Amniotic fluid on amniocentesis or amnioscopy and at ARM. (11-19)

An analogue of Hounsfield unit in CAT scan is not established in Ultrasonography till date. Time has to answer whether such parameter(s) will revolutionize interpretation of Ultrasound imaging. Several authors have studied differentiation of exudates and transudate based on echogenicity (20) and indices comparing nearby tissues or organs subjectively. An Objective assessment of echogenicity has been evaluated in screening of ovarian tumors and studies on PCOS (polycystic ovarian syndrome)

using Image J analysis (21,22). In this study the same image J software was used for estimation of AF echogenicity and correlated with the turbidity.

Figure 1

Mechanism of Labor in terms of amniotic fluid particles, echogenicity and turbidity. (9,23-26). PG- Prostaglandins. PGHS – Prostaglandin H synthase. IL- Interleukin. TNF- Tumor Necrosis Factor. MMP- Matrix Metalloproteases. CRH- Corticotropin Releasing Hormone. HSD - Hydroxy steroid dehydrogenase. NF- κ B (nuclear factor kappa-light-chain-enhancer of activated B cells). Green arrows shows stimulation.



MATERIALS AND METHODS

This observational study comprises 41 women with Singleton pregnancies who had third trimester scan on routine antenatal visits and image J analysis of USG images. The Echogenicity index was estimated based on principle of comparing the echogenicity of AF with surrounding tissues including placenta and fetal parts. Image J software (public domain – National institute of Health downloadable at rsb.info.nih.gov/ij/index.html) was used for analysis. Three images preferably in different quadrants were analyzed at a time and an average is taken. Mean echogenicity of amniotic fluid pocket alone without cord structures were calculated first after digitization of ultrasound image. Later the surrounding structures were included in (ROI) Region of interest. The ratio was obtained between the former and later and termed as echogenicity index was expressed in percentage. Hyperechoic foci from the surrounding background and fetal bone regions were excluded in the mapping. Image gain parameters were adjusted identically. The last reading was obtained at ARM in spontaneous onset of labor and correlated to AFOD 650nm. Measurements were taken when AFI was in normal range. Meconium and blood

stained amniotic fluid samples were excluded. Cases of Chorioamnionitis were excluded. This study confirms to standards of declarations of Helsinki.

Images illustrating principles of measurement of echogenicity.



Figure 2a

Echogenicity index = mean echogenicity of Image 1/2
 $78.64/101.55 \times 100 = 77.8 \%$

Results				
File	Edit	Font	Results	
	Area	Mean	Min	Max
1	29996	78.645	36	232
2	183052	101.552	36	240

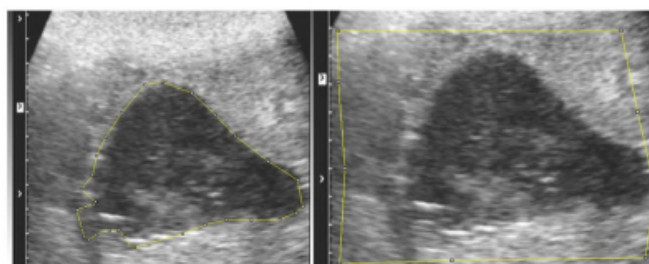


Figure 2b

Echogenicity index = mean echogenicity of Image 1/2
 $79.48/115.379 \times 100 = 68.88 \%$

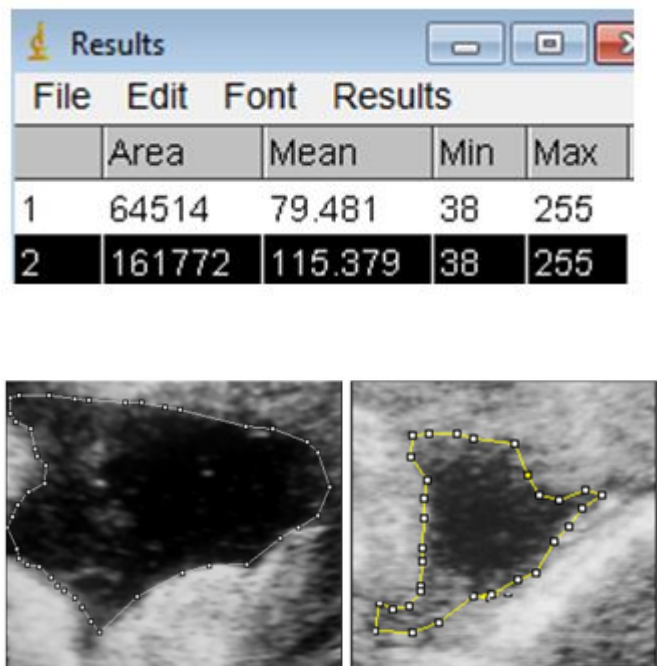


Figure 2c

Low echogenicity index which is evident of selected region of interest 38.7 % and 51.2 % respectively.



OBSERVATION AND RESULTS

Figure 4

A rapid increase in amniotic fluid echogenicity is noted after a value in the range of 60-65 % percent (n=13) and onset of Labor is taking place at around 0.70.

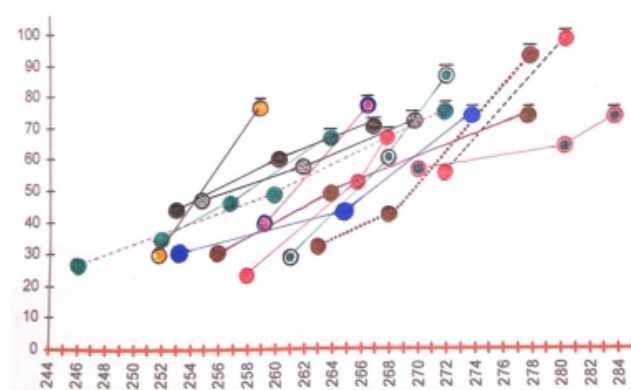


Figure 5

Comparison of naked eye appearance of amniotic fluid collected in test tubes with labeled AFOD at 650 nm.

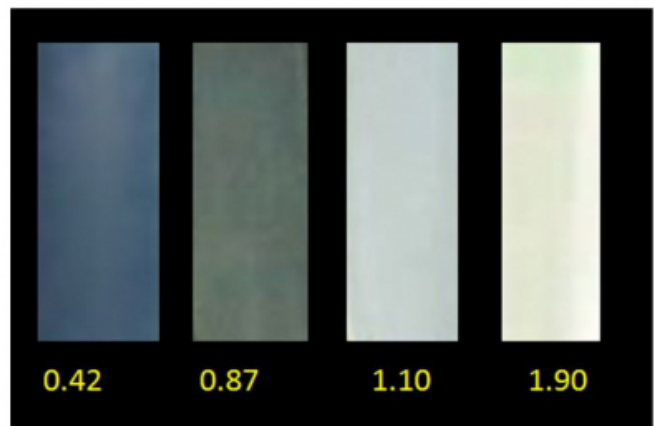


Figure 6a,6b

Illustration showing Serial echogenicity pattern in third trimester prior to onset of labor with simultaneous increase in AFOD values.

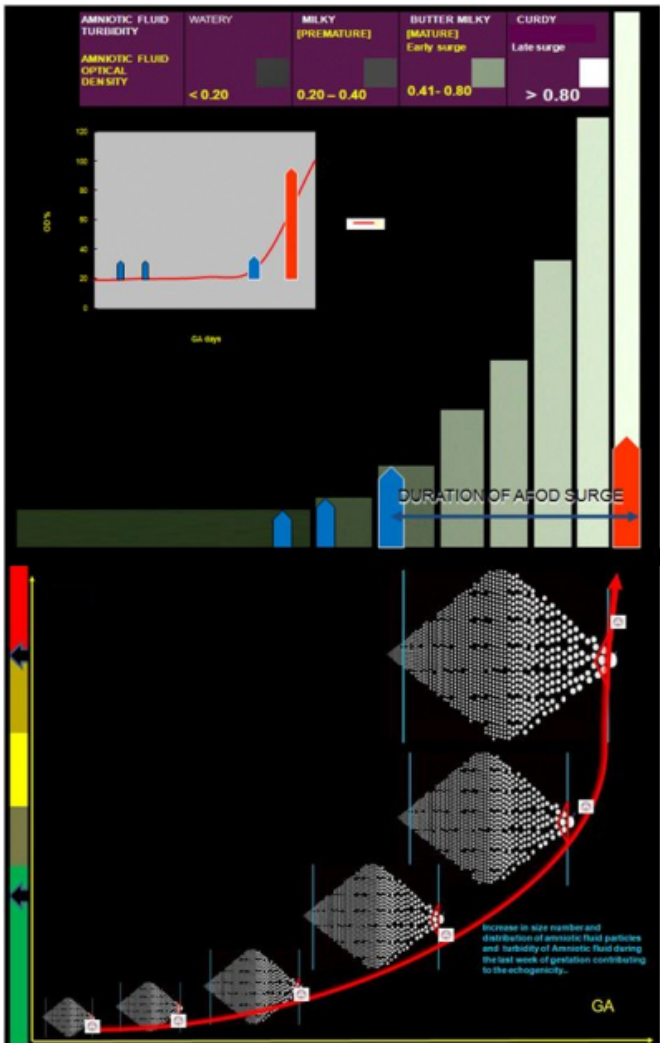


Figure 7

Picture of USG images of high echogenicity index more than (70 %) in the last week of pregnancy. A rapid increase in echogenicity index in late third trimester indicates rise in amniotic fluid optical density and predicts labor. These images are indicators of imminent labor.

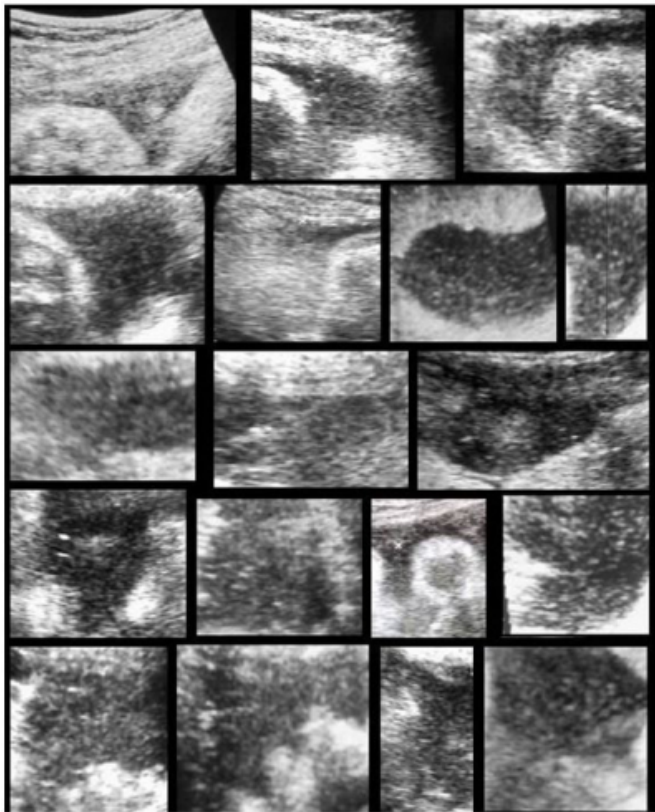


Figure 8

Picture showing naked eye appearance of particles demonstrated in bag of membranes while doing LSCS. The optical density was 1.40 with echogenicity index of 73 percent.

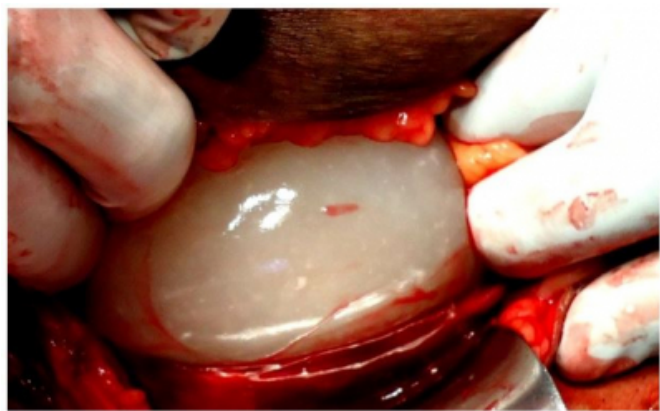


Figure 9

Graph showing correlation of amniotic fluid echogenicity with respective AFOD 650 values (N=33). Mean AFOD of amniotic fluid samples collected at ARM at spontaneous onset of Labor was 1.21SD0.33 and echogenicity index was 73.15% SD 8.5. Min AFOD were 0.72 and max value of 1.92. Echogenicity index ranged from 62 to 93%. The graph shows linear and compound trend lines with significant positive correlation ($p<0.05$)

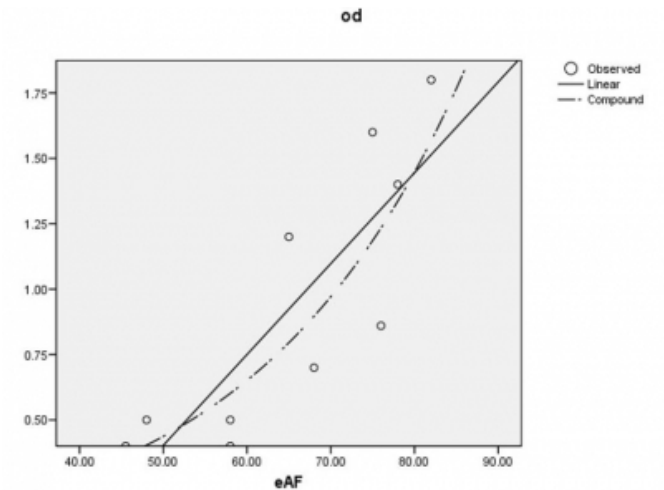
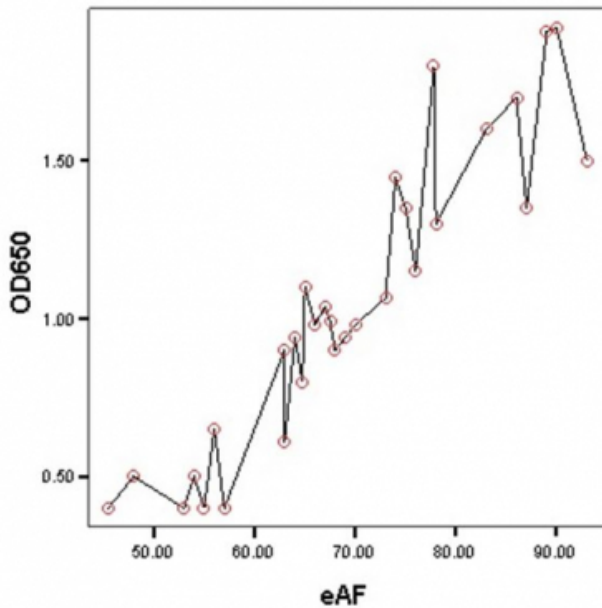


Figure 10

Mean AFOD at delivery was 1.06 ± 0.42 and echogenicity index was $69.40 \pm 11.09\%$ (N= 41) when cases of PROM with low AFOD values were included for purpose of plotting the graph and correlation with echogenicity index. (min AFOD were 0.4 and max value of 1.92). Graph showing correlation of amniotic fluid echogenicity with respective AFOD 650 values. Echogenicity index ranged from 45% to 93%. None of the babies had RDS. There was no mortality in this series. The mean Gestational age was 38 weeks 4 days.



DISCUSSION

Echogenicity of amniotic fluid indirectly represents size number and distribution of amniotic fluid particles and in turn turbidity of amniotic fluid. The mean optical density at onset of labor is around 1 at 650 nm during the rapidly increasing phase of turbidity. (1, 2) We infer that it corresponds to mean echogenicity of about 67 %. When values beyond this were attained in late third trimester, onset of labor takes place within week duration.

Zabkar et al study on amnioscopy and changes in liquor clearly mentions the significance of turbidity of AF and onset of labor. Delivery began spontaneously with appearance of milky amniotic fluid although cervix was not yet ripe and although the cervix was ripe the delivery did not begin spontaneously if the amniotic fluid was clear. The nuances of milky amniotic fluid begin to be observed approximately 7 days before spontaneous delivery. However the study does not give importance to EDD just based on gestational age alone. It proposed that every woman has her calculated term (or unknown), the term at which she delivers

and the term which would be most favourable for the infant to be born. The end point of gestation and optimum maturity was taken as mature liquor. It highlights that inductions should be started when milky mature) amniotic fluid appears if delivery does not start spontaneously within some days (13).

With advent of USG the GA assessment has become more quantitative and horizontal, neglecting the vertical growth as described by Klimek who validated the pregnancy as Independent time spatial event – The concept of individual term. With dating by USG there still remains a 6 weeks confidence interval of EDD. The consenses of routine induction at a particular gestational age based on USG alone need retrospection. The interval can be reduced further with estimation of serum oxytocinase, hyaluronic acid levels (27-31). Amnioscopy is used in Salings institute for post dated pregnancy (15, 17) and as fetal surveillance tool. Also flexible fibre optic amnioscopy is done (16).

Several surges take place during the last week of gestation. The changes in surfactant levels, L/S ratio, cortisol, prolactin, PGF2alpha, relaxin show similar pattern of increments (19, 31-33). The changes in amniotic fluid macroscore (based on cloudiness and amount of flakes in AF obtained by amniocenteses) undergo the same exponential pattern as shown by Verpoest et al (12). The macroscore correlated significantly with AF OD 750 nm. Earlier the delivery, the earlier occur the increase in macroscore. The score increased in days before delivery regardless of the duration of Gestation. The faster maturing children will be delivered earlier and the slower ones later. That's the reason for the appearance of amniotic fluid has inter-individual variation at a crosssection of Gestational age. Retrospectively klimeks hypothesis of individual term has been proved by Zabkar and Verpost. Thus identifying the amniotic fluid optical density less invasively shall promote a successful, appropriate and safe obstetric practice.

In our study the change in echogenicity of amniotic fluid could be measured quantitatively towards the end of pregnancy. Labor takes place from 36 to 41 weeks of gestational age in Indian subcontinent. We infer that an echogenicity of more than 70 % (approximately more than two thirds) on serial estimation predicts onset of labor with high probability within a week. In a study by Chawanpaiboon et al showed that presence of FFPs and a BPD of more than 93mm (a gestational age of above 34-36 wks in our study population) has likely hood of L/s ratio of more than 2 and the lungs are mature (34-35). In a similar

study by shibata et al (36) inferred that about 82% of babies were delivered within less than a week after echogenic amniotic fluid was detected though it was not quantified, actually represents the surge in AFOD values. The computer assisted analysis in our study is one step further in understanding the relevance of echogenicity of AF. It is during the surge the preparation of labor (countdown) takes place which we can monitor sonographically. Thus serial eAF values guide in programming and institutionalizing labor. The sequence of events; viability, maturation of fetus, preparation & onset of labor takes place in each pregnancy. There is also increase in levels of maternal clotting factors near labor (37, 38). The initiation of labor ideally should take place at the

physiologically ripe environment for the fetus as well as mother rather blindly at the convenient hour of Obstetrician. Further advancements in the imaging modalities (7, 39) and studies in future should be able to pinpoint the changes and transabdominally see through the fluids better.

CONCLUSION

Amniotic Fluid echogenicity index (eAF) is helpful to predict onset of labor and can be considered as labor marker. Further studies and advancements in Ultrasonography and image analysis softwares widen the applicability and combining with other labor markers and modalities of fetal surveillance will contribute in optimizing labor.

References

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