Original Research Article

Amniotic Fluid Optical Density (AFOD) correlates with the lung maturity as well as complete maturity of the fetus – A clinical observational study

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Abstract

According to ACOG committee, complications of non-medically indicated (elective) deliveries between 37 and 39 weeks are faced with increased NICU admissions, transient tachypnea of the newborn (TTN), respiratory distress syndrome (RDS), increased need for ventilator support, increased sepsis rates (suspected or proven) and higher incidence of newborn feeding problems and other transition issues. Cesarean sections done at less than 39 weeks gestation have increased risk of neonatal morbidity including respiratory distress, hypoglycemia, sepsis, NICU admissions, hospitalization for more than 5 days, etc this study aims at correlating fetal maturity with amniotic fluid optic density.

Key words

AFOD, Amniotic fluid optical density, Lung maturity, Fetal maturity.

Introduction

One of the most important preventive measures in obstetrics is the individual evaluation of the most appropriate time to terminate a pregnancy [1]. It is a conventional decree that babies born between 37-40 weeks of gestation are completely mature [2, 3]. We anticipate the problems of prematurity in those born before 37 weeks which include respiratory distress syndrome, retinopathy of prematurity, Intra-ventricular hemorrhage, periventricular leukomalacia, brain disorders such as neonatal encephalopathy, cerebral palsy, necrotizing enterocolitis, fetal -to-maternal hemorrhage, hyperbilirubinemia, fetal cardiac arrhythmias and that there would be complications of post maturity in those born after

42 weeks [4]. However, it has been observed that at least 3% of preterm births occur beyond 37 gestational calendar weeks [4]. Even after 40 weeks "full term" an incidence of 0.25% RDS has been recorded [5]. In one large study, about 0.05% who were delivered electively between 37 and 40 weeks required mechanical ventilation [6]. Currently, 10-18% of labors are induced prematurely only because the calendar time of pregnancy duration has exceeded 287 or 294 days from the date of the last menstrual period, which is given by the mother [7]. So, is our concept of "term", completely "term"?

However, fetal maturation is a time-spatial process which means that from the increase of spatial parameters one can calculate the individual time of maturation expressed in the number of technical quanta [8] and gestational age is a biologic continuum and new data reveal important insights into the outcomes of babies born during this 5-week period called term [8]. Both fetus and mother in the last 3-4 days of pregnancy undergo rapid pre-labor changes necessary to delivery and sudden adaptation of fetus to extra uterine life. Labor which starts ≥ 1 week before its own individual term (preterm) as well as delayed due to disturbance of its initiation (postponed pregnancy) is possible in the large six-week range (37 – 43). Maturity is neither mass nor time and cannot be assessed in grams or units of time [9]. Therefore modern neonatologists and obstetricians successfully worked out maturity quantizing in points without taking into account mass, length or gestational age of fetuses and newborns [10].

As discussed later, amniotic fluid turbidity, the objective measure of which is Amniotic Fluid Optical Density (AFOD) correlates with the lung maturity as well as complete maturity of the fetus when various studies have been inferred. In this study we set out to find the AFOD retrospectively, at birth (at spontaneous onset of labor/ LSCS) and its correlation with the variables used to define "term" namely, gestational age, birth-weight of the newborn and the neonate's functional maturity.

Surfactant

The internal surface of the alveolus is covered with a thin coat of fluid. Water in this fluid has a high surface tension which promotes collapse of the alveolus. Pulmonary surfactant decreases the surface tension of water, increases lung compliance and prevents collapse of alveoli during expiration [1].

The timing of surfactant production in quantities sufficient to prevent atelectasis depends on an increase in fetal cortisol levels that begin between 32 and 34 weeks. By 34 to 36 weeks, sufficient surfactant is produced by the type II cells in the lung secreted into the alveolar lumen, and is excreted into the amniotic fluid. The concentration of lecithin in amniotic fluid indicates fetal pulmonary maturity. Because the amount of lecithin is difficult to quantify, the ratio of lecithin (which increases with maturity) to sphingomyelin (which remains constant during gestation) (L/S ratio) is determined. L/S ratio of 2:1 usually indicates pulmonary maturity. The presence of phosphotidylglycerol, also is indicative of fetal lung maturity and useful in maternal diabetes [1].

Respiratory distress syndrome (RDS)

A deficiency of surfactant results in atelectasis decreased functional residual capacity, arterial hypoxemia, and respiratory distress. Surfactant synthesis may be reduced as a result of hypovolemia, hypothermia, acidosis, hypoxemia, and rare genetic disorders of surfactant synthesis. These factors also produce pulmonary artery vasospasm, which may contribute to RDS in larger premature infants who have developed sufficient pulmonary arteriole smooth muscle to produce vasoconstriction. Surfactant deficiency – induced atelectasis causes alveoli to be perfused but not ventilated which results in a pulmonary shunt and hypoxemia. As atelectasis increases, the lungs become increasingly difficult to expand, and lung compliance decreases. As the chest wall of the premature infant is very compliant, the infant attempts to overcome decreased lung compliance with increasing

inspiratory pressures, resulting in retractions of the chest wall. The sequence of decreased lung compliance and chest wall retractions leads to poor air exchange, an increased physiological dead space, alveolar hypoventilation, and hyper-capnia. A cycle of hypoxia, hypercapnia and acidosis acts on type II cells to reduce surfactant synthesis and in some infants, on the pulmonary arterioles to produce pulmonary hypertension [11].

Aim and objectives

- To establish correlation between Amniotic fluid Optical density (AFOD), gestational age birth weight and functional maturity of the newborn.
- To obtain mean AFOD at spontaneous onset of labor.
- To study the functional maturity of the newborns, especially in terms of lung maturity by means of presence or absence of RDS in babies born out of spontaneous labor and in those born by elective termination.

Material and methods

Two hundred and two pregnant women were selected for the study.

Inclusion criteria

- Women who underwent first trimester scan and crown rump length estimation, or
- Women with regular periods who underwent scan at less than 20 weeks gestation which is in agreement with the gestational age calculated from the last menstrual period

Exclusion criteria

- Blood stained and meconium stained amniotic fluid samples
- Intrauterine growth restriction
- Premature rupture of membranes
- Preterm premature rupture of membranes
- Amniotic fluid index <5 and >25

Informed consent was taken from all women prior to artificial rupture of membranes and before LSCS. Under aseptic precautions amniotic fluid samples were collected while doing amniotomy after 3-4 cm dilatation by an intramuscular needle fitted with a 2 ml disposable syringe. This procedure was done under vision gently by inserting one Sim's speculum, if necessary two, taking care to avoid injury to the presenting part. Amniotic fluid samples were collected at cesarean section after careful incision on the uterus from the bulging membranes. The color and turbidity of fresh uncentrifuged amniotic fluid samples thus obtained was measured subjectively by naked eye inspection and quantified subjectively as per **Table – 1**.

Table – 1: Scoring for amniotic fluid turbidity.

Color/ Turbidity of amniotic fluid	Score
Watery	1
Milky	2
Buttermilk like	3
Curd like	4

The color and turbidity of the fresh uncentrifuged sample was objectively quantified by colorimetry. The measurement of AFOD was done at 650 nm after the reading of control test tube with tap water. Babies are observed for the amount of Vernix on their skin immediately after birth before drying of the baby. Routine protocol for neonatal resuscitation was followed. Birth weights were recorded for all babies. APGAR scores at 1 minute and 5 minutes were obtained. Babies were observed for classical signs of respiratory distress (tachypnea >60 breaths /min, grunting, retraction of ribs, sternum. Suspected cases of distress were resuscitated with bag-mask ventilation with oxygen and referred to NICU for further management. Respiratory distress was graded using Downe's score. Analysis was done using Microsoft excel 2007.

Results

In this observational study comprising 202 pregnant women, 134 were spontaneous

deliveries. Out of these, 7 women delivered on the day of EDD which corresponds to 5.22%, while, 64.9% of the deliveries took place at gestational age between 37 and 40 weeks.11.9% delivered spontaneously before 37 weeks and 16.42% delivered after 40weeks as per **Table - 2**.

Table – 2: Percentages of spontaneous deliveries at different gestational ages.

Gestational age (days)	Percentage of deliveries	Number of deliveries
≤258	11.90%	16
259-279	64.90%	87
280	5.22%	7
≥281	16.42%	22

The mean period of gestation in days for the total number of cases was 268.584±13.895; it ranged from 196 days to 290 days and the median was 269 days. In the non RDS group the period of gestation ranged from 236 days to 290 days with the mean being 270.288±10.680, and the median being 270 days. In the RDS group, the period of gestation ranged from 196 days to 286 days, the median being 251.167±26.502 and the median were 255 days. The mean AFOD for Non-RDS cases (n=184) 1.058±0.364 was found to be significantly higher when compared to the RDS cases (18) 0.22±0.107; p<0.001. The total number of cases with AFOD <0.40 were 18 and all of them developed RDS. The mean birth weight in total number of cases was 2813±41, while it was 2814±428 and 2388±680 respectively in the non RDS and the RDS groups. The mean birth weight (grams) /gestational age (in days) ratio for total cases was10.455±1.384. The birth weight/ gestational age for non RDS group was ranged from 6.14 to 15.679 the mean being10.429±1.664, and the median 10.467. For the RDS group, it ranged from 9.504 to17.073, the mean was 9.466±2.478 and the median was 9.479.There was no statistically significant difference in birth weights adjusted to gestational age between the non RDS and RDS groups (p=0.0827) as per **Table – 3**. The 202 cases were divided into groups according to gestational age, for comparison; less than 35 weeks, 35 weeks + 1 day to 36 weeks, 36 weeks + 1 day to 37 weeks, 37 weeks + 1 day to 38 weeks, 38weeks + 1 day to 39 weeks, 39 weeks + 1 day to 40 weeks, and greater than 40 weeks.

Out of the total number of cases of spontaneous onset of labor group of 133 only 1 case developed RDS which was in the least gestational age group, while in the elective termination group, RDS was present in 6 out of 7 groups, including the group with period of gestation >280 days. But, the severity of RDS decreased with increasing gestational age (mild RDS with Downe's score <4, severe RDS with >4 Downe's score) as per **Table - 4**.

Discussion

In spite of great scientific advancement, the secrets behind the gestational age at which spontaneous onset of labor takes place with each pregnancy and complete fetal functional maturity is attained with each fetus is evading the obstetrician. The physiology of onset and progression of labor is undoubtedly multi factorial involving various rate limiting complex sequential inter related and mutually supportive cascades. A minor natural variation at any level can affect the duration of pregnancy. Some women have genetic pre-disposition to deliver pre term due to differences at molecular level. Non-infected "preterm" cervical ripening is an inflammatory process like that of term labor. Polymorphisms in several genes regulating cytokines, genetic susceptibility to infections of low virulence, mutations of collagen synthesis, oxytocin receptors, BMI, parity and age are also involved. These factors vary from race to race and also between each feto-maternal unit, resulting in physiological variation in duration of pregnancy.

In the current study between any two groups among 3 to 7 there is no radical change in mean AFOD values when cases of RDS were included for analysis and remained same after adjusting for birth weights. All the babies born with AFOD value around the mean 1.138±0.103 SD were

fully functionally mature. Their skin was mature, pink with very little vernix caseosa adherent to the surface. None of the babies developed RDS when AFOD was more than 0.40. On the other hand, babies born with AFOD value <0.40 (n=18) were functionally premature and developed varying degrees of RDS and had birth weights ranging from 2300 to 3000 g. Their skin was premature, thin, shiny and red in color with plenty of vernix caseosa adherent to the surface. The largest AFOD value below 40 at which babies developed RDS was 0.40. Initially the studies of optical density were done with centrifuged samples. The cut off was determined to be 0.15. The values are variable depending on centrifugation speed and time. The uncentrifuged samples with OD around 0.40 on centrifugation at 2000 rmp for 10 min gives the OD reading around 0.15. We observed that Babies born with AFOD value < 0.40, at a lower gestational age developed moderate to severe and prolonged RDS as compared to babies born with AFOD value <0.40 at a later gestational age who developed milder RDS for shorter duration. The findings are in accordance with the previous studies.

Table – 3: Characteristics of AFOD and birth weight in non RDS and RDS groups compared with total study population.

	Total	**Non RDS**	**RDS**
No. of cases	202	184	18
Mean period of gestation(days)	268.584±13.895	270.288±10.680	251.167±26.502
AFOD mean ±SD	1.058±0.364	1.138±0.103	0.22±0.107
(n)AFOD <0.40	18	0	18
Mean Birth weight(grams)±SD	2813±417	2814±428	2388±680
Birth weight (grams)/ Gestational age (days)±SD	10.455±1.384	10.429±1.664	9.466±2.478

Table – 4: Distribution of cases with and without RDS in different groups of spontaneous onset of labor and elective termination of pregnancy.

	Spontaneous onset of labor			**Elective termination**		
Gestational age (in days)	**Total**	**Without RDS**	**With RDS**	**Total**	**Without RDS**	**With RDS**
<246	3	2 (66.67%)	**1 (33.33%)**	9	3 (33.33%)	**6 (66.66%)**
246-252	10	10 (100%)	0 (0%)	2	0 (0%)	**2 (100%)**
253-259	4	4 (100%)	0 (0%)	4	4 (100%)	0 (0%)
260-266	22	22 (100%)	0 (0%)	15	12 (80%)	**3 (20%)**
267-273	33	33 (100%)	0 (0%)	17	16 (94.11%)	**1 (5.88%)**
274-280	39	39 (100%)	0 (0%)	13	10 (76.92%)	**3 (23.07%)**
>280	22	22 (1000%)	0 (0%)	8	6 (75%)	**2 (25%)**

AFOD represents indirectly the amount of surfactant. Hence severity depends on the how low the AFOD value is or in other words how low the surfactant phospholipids are and not always how low the chronological age is. There could be other factors accounting for less severity of RDS like trial of labor or unknown constitutional factors at advanced gestational age despite low surfactant levels as revealed by low AFOD values. However the severity could be more than expected on rare occasions.

Though 0.40 AFOD value is just enough to prevent RDS, fetal maturity is complete only when all systems attain complete functional maturity. Prematurity in GI system results in

necrotizing eterocolitis, in respiratory system results in RDS, and finally skin results in failure to maintain temperature. Complete maturity of the babies is seen with the AFOD value around 1.

AF Lecithin levels increase from 43 micro grams/ml at 34-35 wks gestation to 147 microgram/ml at term before labor. Further its levels are known to increase up to232 micro grams/ml at term labor. There is a surge in sebaceous gland hyperplasia producing sebum, which is a primary constituent of vernix caseosa coupled with the desquamation of fetal corneocyts during the last trimester. This vernix separation has major contribution to increase AFOD during third trimester. The lung matures parallel over the same period. At higher OD values there were less amount of vernix on body surface of new born. The inverse relation to AFOD values is proved in this study.

The skin maturation follows lung maturity resulting in complete functional maturity of fetus. Considering skin maturity as reliable endpoint, prematurity, optimum maturity and post maturity are part of a spectrum which can happen at any time within 36 to 42 weeks GA during which birth is taking place. Sometimes a failure or delay of mechanism of initiation of labor can result in post maturity as revealed by post mature skin changes seen at high AFOD values > 1.75 which was noted in 4.3% of study population. At a particular day of pregnancy at chronological "term" there could be fetuses that are in the process of obtaining complete functional maturity. Conversely a fetus which is functionally mature at 36 weeks becomes post mature if the delivery is delayed. The understanding of AFOD prevents iatrogenic prematurity as well as complications of post maturity thereby optimizing labor.

Levels of pro labor cytokines like IL-6, IL-8, IL-1 beta, epidermal growth factor in amniotic fluid and maternal serum which are elevated during the progress of labor are produced by the human amniotic fluid cells which are shed by the fetal skin. The onset of labor is more closely related to fetal functional maturity than either gestational age or birth weight. The gestational age at delivery is unique to each feto-maternal unit. This concept of individual term was introduced by Klimek (Let man be born at his own due time). There are gestational age and birth weight independent scoring systems and computer aided methods to know the functional or biological gestational age of the fetus. Klimek and Ballard scoring systems are the commonly used for the postnatal assessment of Gestational age. The child survival and safe motherhood programme guidelines in India advocate that new born without any signs of illness can be managed at home with special care even if the birth weight is as low as 1800 g.

The significance of AFOD is not only to know about lung maturity, but, much of importance lies in defining events taking place beyond lung maturation which include skin maturity and onset of labor. Studies have shown that induced (non-spontaneous) labors have higher Oxytocin requirements and increased incidence of PPH. Presuming the inductions as inappropriate in time, the iatrogenic complications and suffering could have been probably avoided if the inductions were AFOD guided. Inductions just based only on chronological age of the fetus might be the reason for requirement of heavy doses of prostaglandins, dysfunctional labor prolonged induction delivery intervals, more number of instrumental deliveries, pain and larger doses of narcotic analgesia, neonatal respiratory depression, perineal tears and increased caesarean section rates. Such maternal and neonatal morbidities are the result of mechanization of natural process of labor. Induction of labor needs to be reviewed in light of AFOD values or its equivalent indicators which determine the preparedness of labor and hence the biological gestational age that is more relevant than "EDC" (Estimated Date of confinement) which itself is a misnomer.

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One study observed that the delivery of a mature newborn in most cases starts spontaneously at the

moment we recognize milky amniotic fluid by amnioscopy [12]. Many studies convincingly prove that each feto-maternal unit matures at its own time and that this time is irrespective of any single parameter considered as of now. One study inferred that complete functional maturity and onset of labor takes place at individual term with mean AFOD around 0.98 irrespective of birth weight and gestational age, which is taken as the basis for this work.

The maturation of the fetal lungs, that is to say the adequate production of surfactant in the fetal alveoli, as it is well known reaches to its end about the 35 - 36th week of gestation. Even more closely to the date of delivery, the maturation of the fetal skin takes place, i.e., the detachment of the Vernix caseosa from the fetal skin into the amniotic fluid. This means that the fetuses with "mature skin" should have mature lungs too. This has been proved by the comparison of the values of the L/S Ratio (as a criterion of the lung-maturity), to those of the turbidity of the amniotic fluid [9]. Studies using in vitro analysis have shown that the interaction between pulmonary surfactant and vernix caseosa could explain the appearance of amniotic fluid turbidity. That phenomenon is interpreted based on the "roll-up" hypothesis [13].

Pulmonary surfactant and vernix caseosa complexes in swallowed amniotic fluid might locally influence fetal intestinal enterocytes [14]. Vernix is rich in nitrogen containing amino-acids such as glutamine and asparagines [15]. Glutamine has received increased attention as a possible trophic factor for the preterm infant and has been implicated in gut maturation [16]. Findings elucidate the physiological interactions among pulmonary, dermal-epidermal, and gastrointestinal developmental processes [10].

During the third trimester of human gestation, there is a progressive increase in the turbidity of the amniotic fluid surrounding the fetus [17]. The precise etiology of amniotic fluid turbidity is potentially multifactorial. Amniotic fluid turbidity has been related to an increase in lung derived phospholipids and to the presence of lamellar bodies derived from type II pnemocytes [17]. Turbidity primarily results from detachment of vernix caseosa from the fetal skin surface. In one study the rheological properties of veernix were assessed as a function of physiologic temperature, indicating a potential mechanism for inducing amniotic fluid turbidity and suggest a novel physiolologic interaction between the skin and lung during late fetal development [18].

Late in the second trimester, and particularly in the third trimester, the sebaceous glands become large complex structures [18]. Sebum, the end product of sebaceous gland secretion, forms a primary constituent of vernix caseosa [18]. Vernix also contains desqumated fetal corneocytes indicating a contribution from terminal differentiation of the epidermis [18]. The lung develops and matures over the same time [18].

Amniotic fluid is either clear or turbid at the time of amniocentesis, and this turbidity has been correlated with maturity by many authors. Based on a visual comparison between mature and immature unspun amniotic fluid specimens, a correct distinction (confirmed by biochemical markers) was recorded 87% of the time. One author reported a similar association based on the ability to read newsprint through a test tube containing amniotic fluid. The inability to read print through amniotic fluid correlated with mature lungs. Many investigators have shown an association between amniotic fluid turbidity assayed at 650 nm and fetal maturity [18].

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