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Accelerated Clotting Time with Amniotic Fluid: A Reliable Indicator of Fetal Maturity

Chitra S, Pralhad Kushtagi, Kuntal Rao

Abstract

Estimation of gestational age is one of the corner stone of prenatal care. Preterm birth rates are higher in developing countries. Women presenting late in pregnancy poses a great problem in assessing gestational age, even USG may not provide correct estimation. Amniotic fluid near term contains an increased amount of surfactant protein. It accelerates the clotting if added to the blood. Accelerated clotting time (aACT) in amniotic fluid is a simple easy bedside test especially for unreported cases admitted with uncertain gestational length.

Key words: Amniotic fluid; Clotting time; Preterm.

No.B-2(s), X-A, Cross Thillai Nagar, Tiruchirapally - 18
Chitra S, DGO, MD Obstetrician-Gynecologist

Kasturba Medical College and Hospitals, Manipal University,Mangalore 575001, India
Pralhad Kushtagi, MD DNB FICOG Professor in Obstetrics-Gynecology

Faculty of Medicine and Health Sciences, Sultan Qaboos University, Al-Khoud, Oman
Kuntal Rao, MD Senior Consultant in Obstetrics-Gynecology

Correspondence to:
Dr. Pralhad Kushtagi, Email: pralhadkushtagi@hotmail.com

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Introduction

Estimation of gestational age and fetal maturity is one of the cornerstones of prenatal care. The rate of admissions of neonates to neonatal intensive care units because of prematurity has remained almost unchanged, despite the availability of ultrasonography in prenatal care. Preterm birth rates are estimated to be substantially higher in developing countries [1].These figures appear to be on the rise [2]. Systematic analysis of preterm births since 1990 has shown that more than 60% of preterm babies are born in south Asia and sub-Saharan Africa, where 52% of the global live births occur [3].

Inability to provide correct menstrual history on the part of the mother contributes to 10-45% of such admissions [4]. In most health care facilities in India, it is not uncommon to find women presenting late in the last trimester for a check-up or sometimes directly in labor. Although the date of last menstrual period is not in itself a very reliable method for calculating gestational age [5], even ultrasonography at this stage will not provide the correct estimation of duration of pregnancy [6]. There is a need for a test that can provide reliable information about fetal maturity in situations where the duration of index pregnancy is not known. The shake test [7], spectrophotometric optical density reading at 650 nm wave length [8,9], Nile blue sulphate stained fat cell measurement and estimation of accelerated clotting time (aACT) in amniotic fluid [10] are some of the investigations used for determining fetal maturity under such circumstances.

aACT was studied as a test for determining fetal maturity in the period between 1975 to 1985. It was reported as equal to [11] or better [12] than the available methods to determine gestational age but it did not gain popularity.

The basis of aACT is that amniotic fluid from near term pregnancy

contains an increased amount of surfactant proteins and desquamated degenerated fetal cells [10]. These contribute to elevated phospholipid and thromboplastins in amniotic fluid. If such amniotic fluid is added to blood, it will initiate an extrinsic coagulation cascade and accelerate the clotting. The amniotic fluid of preterm pregnancies with a lesser amount of surfactants and desquamated cells results in no effect on clotting time on its addition to the blood.

Even today the facility of ultrasonography, especially trained sonologists and fetal biometric profiles for the local populations are not available at all health care centers, especially in rural areas. When faced with pregnancy with uncertain gestational age in labor, there is a need for a simple bedside test for the clinicians working in such remote areas that can help them to identify possible preterm births.

The present report is an effort to revive the interest in amniotic fluid studies by validating aACT against other fetal age determining tests from amniotic fluid.

Materials and Methods

Consecutive consenting 100 laboring women admitted with singleton pregnancy presenting by vertex during the study period of 6 months were recruited. History of the last menstrual period and ultrasound determined age if done in first half of pregnancy was noted.

Amniotic fluid (6-8 mL) was collected at low amniotomy in active labor or at admission if the membranes had already ruptured. Samples with blood or meconium contamination were excluded from the study.

Amniotic fluid was used for determination of accelerated clotting time, optical density at 650 nm by spectrophotometer, creatinine concentration and for performing the rapid bubble test (as described by Clements *et al*, 1972) [7].

For the interpretation,a value of 1.7 mg/dL for creatinine concentration [8, 9] and 0.15 for optical density[13] at 650 nm were taken as the cut-off. The results above the cut-off were considered as positive, indicating fetal maturity, and those below as negative.

The rapid bubble test was performed using only up to 1:1 dilution. Based on the presence or absence of stable foam at the meniscus in both the test tubes after 15 minutes, the test results were grouped as positive (mature) or negative, respectively.

For determination of accelerated clotting time, 1.5 mL of fresh amniotic fluid was mixed with 1 ml of freshly drawn maternal venous blood in a plastic test tube. The test tube was placed in a water bath maintained at 37^0 C and tilted every 5 seconds till complete clotting was observed and the time taken was noted as accelerated clotting time. Clotting time of less than 110 seconds was considered as positive and indicative of fetal maturity [10].

Creatinine estimation and spectrophotometric analysis was done by laboratory personnel who were not aware of gestational age; the bed-side rapid bubble test (RBT) and determination of aACT was carried out by the single person (SC). The results of amniotic fluid analysis were not disclosed to the treating obstetrician until completion of the study.

A modified Dubowitz scoring system was used to rate newborn maturity and assess gestational age.

Student's *t*-test was used wherever necessary to note the significance of difference in the averages. *P* values of less than 0.05 were considered as statistically significant. The sensitivity, specificity and predictive values of various tests in amniotic fluid with a cut-off determined age for respective tests was analyzed with reference to the gestational age of the neonate by newborn maturity rating (NMR).

Results

Amniotic fluid from 100 laboring women was used for determining accelerated clotting time,

optical density at 650 nm by spectrophotometer, creatinine concentration and for performing the rapid bubble test. Eighty-eight percent of the recruited were primigravidas and were aged between 20-30 years; 69% had uncomplicated pregnancies and 13% did not recall their last menstrual date.

Based on NMR, there were 21 neonates grouped as 36 weeks or below. Among them, one case of transient tachypnea was noted with no cases of respiratory distress syndrome. Other complications recorded were hypothermia in 3 (14.2%), hyperbilirubinemia in 2 (9.5%) and hypoglycemia in one case (4.7%). Among 79 term babies, two neonates developed hypoglycemia (2.5%) and 8 had hyperglycemia (11.2%) during the postnatal stay.

Eighty-seven women provided their last menstrual period and it did not correlate with NMR in 18 of them. The menstrual age underestimated the gestational age in 10 and overestimated in 8 cases.

Ultrasound reports before 20 weeks of pregnancy were available with 71 women. The gestational age indicated by ultrasound examination when compared with NMR corresponded (± 2 wk) in 63 cases (89%). The estimated sonological age was less in 8 (6%) and more in 2 (3%) cases.

The creatinine concentration tallied with NMR in 76 cases (58 term, 18 preterm). In 3 cases, it was false positive and false negative in 21.

There were 71 cases classified as term based on optical density at 650 nm by spectrophotometer. In one of the cases with preterm delivery with renal failure, it was 0.53.

Table 1: Accelerated clotting time with amniotic fluid at different gestational age

Gestational age (weeks)	Cases	aACT (sec)		t-value/ significance
		Mean	SD	
31	2	215.0	7.1	5.4/ <0.001
32	3	196.7	28.9	5.2/ <0.001
33	4	163.8	46.4	3.5/ <0.01
34	2	155.0	21.2	2.9/ <0.05
35	2	145.0	21.2	2.5/ <0.05
36	8	112.5	20.7	NS
37	8	83.1	32.9	
38	11	82.7	51.4	
39	24	65.4	31.3	
40	26	51.3	50.1	
41	10	36.5	14.5	

aACT= accelerated clotting time with amniotic fluid; SD=standard deviation; NS= not significant

Table 2: Reliability of various tests with amniotic fluid for fetal maturity

	Fetal maturity test (cut-off values)			
	Creatinine (1.7 mg/dl)	OD 650 nm (0.15)	Rapid Bubble Test	aACT (110 sec)
True positive (n)	58	71	64	74
False positive (n)	3	1	4	2
True negative (n)	18	20	17	19
False negative (n)	21	8	15	5
Sensitivity (%)	73.4	89.9	81	93.6
Specificity (%)	85.7	95.2	80.9	90.4
PPV (%)	95.1	98.6	94.1	97.3
NPV (%)	46.1	71.4	53.1	76.1

OD=optical density; aACT=accelerated clotting time with amniotic fluid; PPV=positive predictive value; NPV=negative predictive value

The optical density was lower than 0.15 in 20 preterm and 8 term pregnancies.

The clotting time accelerated on addition of amniotic fluid was found to have an inverse relationship with increasing gestational age based on the last menstruation period or ultrasonographic examination (P<0.001, r=0.7490). The aACT in preterm pregnancies (< 36 weeks) was 112 seconds or more and at or after 37 weeks of pregnancy was below 83.1 seconds(Table 1). Accelerated clotting time was positive (<110 seconds) in 74 term and 2 preterm pregnancies; it was negative in 19 preterm and 5 term cases.

Validation of various tests used on amniotic fluid to know fetal maturity showed that accelerated clotting time determination was more reliable. It was found to have high specifity (90.4%) and sensitivity (93.6%) with a good positive predictive value (97.3%). The negative predictive value was also high (76.1%) in comparison with other studied tests (Table 2).

Discussion

Untimely birth is a major health hazard. When the gestational age is not certain and a woman is admitted in labor, the onus of recognizing an impending preterm birth early in order to provide the best available neonatal care falls on the obstetrician. Relying only on the calculated duration of pregnancy based on the last menstrual period has been questioned [14]. Ultrasonography does provide more accurate information about estimation of expected date of delivery [15], especially when carried out in early pregnancy, but it is not uncommon in rural settings to find women reporting late in pregnancy for an antenatal check or being admitted in labor.

It is reported that the thromboplastic activity in amniotic fluid reflects the overall fetal maturity as these are released in to amniotic fluid near term [10], mainly due to the presence of surfactant lipoprotein [12]. The thromboplastic activity tested as accelerated clotting time not only has a correlation with surfactant titer by the shake test [16] but also provides higher diagnostic accuracy when combined with the shake test [12]. The accelerated clotting time has also been reported to correlate well with the lecithin-sphingomyelin ratio and with the clinical outcome of the newborn [11].

We have not studied the outcome with reference to the occurrence of respiratory distress in the newborn as in other studies [12, 17]. Creatinine concentration in amniotic fluid, its optical density at 650 nm and the other tests do provide some indication about fetal maturity. Of the various tests chosen in the present study, accelerated clotting time showed a higher sensitivity, specificity and positive predictive value than the others. Besides, it has all the advantages of being a cheap, simple, easy to perform test, providing a result within few minutes.

Despite the possible bias that the person performing bedside determination of aACT and RBT could not be kept blinded from some information regarding gestational age, non availability of these results to laboratory personnel and the treating obstetrician adds some authenticity to the conclusions drawn. In pregnancies with fetal growth restriction due to chronic fetal hypoxia, the surfactant appearance may be hastened. Although there were no cases with obvious fetal growth restriction included in the present study, it should be borne in mind that in such cases aACT could be less reliable.

aACT is a simple, easy bedside test that can be performed without a laboratory or the help of expensive instruments. It has the potential to be inducted as one of the necessary tests in the labor room for un-booked women or those admitted in labor with uncertain gestation length at first referral units. It is too early to conclude about the utility of aACT as a useful tool to triage labors that require transfer to a facility with a neonatal intensive care unit. Effectiveness and efficiency studies are required.

Author contributions

Chitra S collected, analyzed and interpreted the data; Kushtagi P interpreted the data, drafted the manuscript, revised it critically for important intellectual content and gave the final approval of the version to be published; Rao K conceived and designed this study and interpretedthe data.

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