

Clinical Effects of Echogenic Amniotic Fluid on Neonatal Outcome of Term Pregnancies / A Case Control Study

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Abstract

Background: Throughout development, the embryo and fetus are surrounded by a clear, yellow fluid called amniotic fluid (AF), which serves a variety of functions. Meconium-stained amniotic fluid occurs in 8-10% of all deliveries. It has been associated at times with suboptimal neonatal outcomes, although this is controversial. Amniotic fluid may be quickly and non-invasively assessed using ultrasound (U/S). It provides a secure real-time alternative with similar therapeutic results. This study aimed to identify the role of U/S in detecting echogenic amniotic fluid and its association with pregnancy outcomes.

Methods: A case-control study was carried out in the obstetrics & gynecology departments at Al-Elwiya (Baghdad) and Bunt Al-Huda (Dhi Qar) Teaching Hospitals. A total of 174 pregnant women who presented with singleton viable pregnancies and decreased fetal movement were recruited and they were classified into two groups according to echogenicity of AF by U/S. The first group included

87 women with positive echogenic AF (Case group) and the second group included 87 women with negative echogenic AF (Control group). A structured questionnaire was designed, and it included maternal characteristics and pregnancy outcomes. U/S was done by two specialists in the department of radiology. Data analysis was done by using version 26 of the Statistical Packages for Social Sciences. Pearson Chi-square test (α -test) was used to examine the significance of the difference between different percentages. A level of P- P-value < 0.05 was considered significant.

Results: Echogenic AF on U/S predicted a much higher proportion of meconium (60.9%) and bloody-stained amniotic fluid (9.2%) compared to controls where only 14.9% had meconium and non-had bloody stained AF, A positive test result had sensitivity and specificity in predicting meconium of 80.3% and 68.5% respectively. In positive echogenic AF and compared with controls, the rate of preeclampsia, cesarean section, neonatal admission to the intensive care unit, and neonatal death were significantly higher, while the mean Apgar score at 5 minutes was significantly lower.

Conclusions: This study concluded that the confidence of U/S was 37.9% in pregnant women with meconium-stained AF, with sensitivity and specificity of 80.3%, and 68.5% respectively. A positive test of echogenic AF on U/S was highly significant in the prediction of neonatal admission to the ICU because of meconium aspiration syndrome. Training obstetrician residents to be experienced in evaluating the echogenicity of amniotic fluid in obstetrical units & labor rooms is recommended.

Keywords: Amniotic fluid, Ultrasonography, Echogenic amniotic fluid, Meconium stained.

Introduction: Amniotic fluid is a clear, yellow fluid that surrounds the amniotic sac throughout pregnancy. It serves a variety of functions and envelops the embryo and fetus during development. In the case that there is harm to the mother's abdomen, it physically shields the fetus. Additionally, it lowers the danger of compression between the fetus and the uterine wall by acting as a cushion between the fetus and the umbilical cord ⁽¹⁾. Because amniotic fluid naturally has antibacterial qualities, it also aids in shielding the fetus from pathogenic pathogens. It also acts as a reservoir for the fetus's fluid and nutrition, including vitamins, proteins, electrolytes, and immunoglobulins from the mother. It supplies the fluid, space, and growth factors required for the fetal organs' appropriate development and growth, including the musculoskeletal, gastrointestinal, and pulmonary systems. Amniotic fluid is a useful tool that clinicians may use to track the development of pregnancy and forecast fetal outcomes ⁽²⁾. The uniform distribution of fluid during labor allows for the early application of force during cervix contractions when the presenting portion is high. The name "meconium" comes from the Greek word mekoni, which means "opium-like" or "poppy juice." This term refers to the idea, typically credited to Aristotle, that meconium exposure during pregnancy would cause newborn sadness or lethargy. It is the first material that the developing fetus has in its intestines and is responsible for the newborn's first bowel movement. Meconium may be yellow, brown, or green in color. Meconium is passed by healthy-

term newborns 24 to 48 hours after delivery. Infants born before term usually show delayed passage⁽³⁾.

Intestinal contractions and the relaxation of the anal sphincter brought on by fetal distress during birth allow meconium to enter the amniotic fluid and pollute it. Meconium passing into the amniotic fluid is more likely in late deliveries and happens in 5–20 percent of pregnancies overall. In less than 5% of cases where meconium is detected in the amniotic fluid, meconium aspiration syndrome occurs. Normal amniotic fluid is clean; however, meconium⁽⁴⁾ may tinge it green. Among the tools most frequently used in obstetrics is ultrasound. details. In clinical practice, it is used for disorder screening, diagnosis, and therapy. It seems to be safe during pregnancy, although fetal exposure periods should be kept to a minimum while utilizing the lowest power output required to get the required data. In clinical practice, it is used for disorder screening, diagnosis, and therapy⁽⁵⁾. Amniotic fluid may be quickly and non-invasively assessed with ultrasound. According to some research, the more straightforward dye dilution procedure can provide more reliable results when assessing amniotic fluid than sonography does. On the other hand, ultrasonography provides a secure instantaneous solution with similar clinical results. Sonographic evaluations might be either semi-quantitative or qualitative. Semi-quantitative techniques with established reference ranges that are often used in clinical practice include the maximum vertical pocket (MVP) and the four-quadrant amniotic fluid index (AFI)⁽⁶⁾. If ultrasonography is used to diagnose echogenic amniotic fluid, the fluid might be meconium, blood, or vernix caseosa. Towards the conclusion of the third trimester, a substance called vernix caseosa—a mixture of sebaceous matter and epithelium—detaches from the fetal skin and enters the amniotic fluid. It appears that in certain fetuses, the layer of vernix caseosa massively detaches, resulting in a peculiar sonographic image that is described as floating, minute, solid particles that are 2-3 mm in length. Vernix caseosa may be associated with post-maturity.⁽⁷⁾

Patients and Methods: This is a case-control study carried out in the obstetrics & gynecology department at Al-Elwyia (Baghdad governorate) and Bunt Al-Huda (Dhi Qar governorate) Teaching Hospitals. The period of the study was from the 1st of July 2019 to the 30th of July 2020. A total of 174 pregnant women who presented with singleton viable and term pregnancies and decreased fetal movement were recruited for this study. All were fully assessed after history examination, investigations, and U/S, then, they were classified into two groups according to echogenicity of amniotic fluid by U/S. The first group included 87 women with positive echogenic AF (Case group) and the second group included 87 women with negative echogenic AF (Control group). Patients who refused to participate or had twin or preterm pregnancies, frank rupture of membranes, and fetuses with congenital anomalies were excluded from the study. U\S used is done by two specialists in the department of radiology and the device used was with a convex transducer frequency of 3.5 MHz (Braun, U.K.). A structured questionnaire was designed for this study, and it included maternal characteristics (age, parity, gestational age, antepartum, and intrapartum) and pregnancy outcome (MSAF, mode of delivery, Apgar score, and admission to neonatal intensive care unit). Assessment of fetal well-being was done for all patients by kick count, calculation of BPP, and non-stress test NST.

If NST is reactive then the woman was kept under observation for fetal wellbeing by kick count, serial ultrasound to assess AFI weekly, BPP, and NST which was done twice weekly, and we decided the time of termination of pregnancy accordingly. If the NST was non-re-assuring then interference by CST was done using intravenous administration of exogenous oxytocin to the pregnant women, starting with 2 units oxytocin in 500ml glucose water and monitor uterine contractions, the target was to achieve efficient uterine contraction (at least 3 in 10 minutes), with continues monitoring of fetal heart rate and variability by CTG. Thus, the mode of delivery was decided according to the BISHOP score and fetal condition. Labor was closely monitored by plotting maternal, fetal condition, contractions, cervical changes, descent of the presenting part, mode of delivery (vaginal delivery or cesarean delivery). Examination and resuscitation of the newborn by the pediatrician was done.

All analyses were performed using SPSS version 25.0 (IBM Corp.). The significance of the difference between different means (quantitative data) was tested using the Students-t-test for the difference between two independent means. The significance of the difference of different percentages (qualitative data) was tested using the Pearson Chi-square test (χ^2 -test) with the application of Yate's correction or Fisher Exact test whenever applicable. Statistical significance was considered whenever the P value was less than 0.05.

Results

This study included 174 pregnant women with singleton pregnancy and decreased fetal movement. They were divided according to echogenicity of amniotic fluid by ultrasound into case group (n= 87) and control group (n= 87). The mean gestational age in the study group was 39.1 ± 1.1 weeks versus 39.8 ± 1.4 for the control group. Regarding gravidity, 45 women (51.7%) in the case group were multigravida and 42 (48.3%) were primigravida, while in the control group, there were 61 (70.1%) multigravida and 26 (29.9%) primigravida.

In comparison between the two studied groups regarding the predictive accuracy for meconium and or bloody stained amniotic fluid observed after membrane rupture, the U/S criteria of echogenic amniotic fluid was significantly associated with the type of amniotic fluid observed at membrane rupture. Echogenic U/S predicted a much higher proportion of meconium (60.9%) and bloody stained liquor (9.2) % compared to the control group with echo-free amniotic fluid on U/S where only (14.9%) had meconium and non-had bloody liquor.

Table (1): The comparison between the two groups according to the type of amniotic fluid.

Amniotic Fluid	Groups		P- Value*
	Positive Echogenic Liquor No. (%)	Negative Echogenic Liquor No. (%)	
Normal	26 (29.9)	74 (85.1)	0.001
Meconium	53 (60.9)	13 (14.9)	
Bloody	8 (9.2)	0 (0)	
Total	87 (100.0)	87 (100.0)	

*Significant difference between percentages using Pearson Chi-square test at 0.05 level.

A positive test result (echogenic AF) had sensitivity of 80.3% and specificity of 68.5% in predicting meconium. In the current study 37.9% of U\S examined pregnant women had meconium observed at membrane rupture, so without knowing any other clue about the pregnant women in our study, one can predict meconium with 37.9% confidence by chance alone. Testing positive (echogenic AF) would increase the confidence in seeing meconium 60.9% only (PPV). A false positive test had probability of 32.5%. Testing negative (echo-free AF) would rule out a possible diagnosis of meconium with a confidence level of 85.1%(NPV). A false negative test result had a probability of 19.7%. As illustrated in (Table 2).

Table (2): Validity of US-detected echogenic liquor in predicting meconium-stained liquor at membrane rupture.

Echogenic Liquor U/S	Meconium Stained		Total
	Positive	Negative	
Positive	53	34	87
Negative	13	74	87
Total	66	108	174

Sensitivity=80.3%, False negative test result= 19.7%, Specificity=68.5%, False positive test result=32.5%, Accuracy=73%, PPV=60.9%, NPV=85.1%.

In this study, preeclampsia was significantly more frequent among the study group compared to the control group (18.4% vs 8.1%, P= 0.047). The incidence rate of emergency C/S was significantly higher among cases with positive echogenic amniotic fluid compared to controls with non-echogenic amniotic fluid by U/S (70.1% vs 12.6%, P= 0.001). Pregnant women with positive echogenic U/S test had a significantly increased risk of having an emergency C/S by 2.9 times compared to the control group. The incidence rate of admission to the neonatal intensive care unit was significantly higher among cases with positive echogenic amniotic fluid compared to the control group (69% vs 20.7%, P= 0.001). The incidence rate of neonatal death was significantly higher among cases with positive echogenic amniotic fluid compared to those with negative echogenic amniotic fluid (6.9% vs 0%, P= 0.023) of the control group. The mean Apgar score at 5 minutes was significantly lower among cases with positive echogenic amniotic fluid by U/S compared to controls with negative echogenic AF (6 vs 8, P= 0.001). As shown in (Table 3).

Table (3): The comparison between the two groups according to related risk factors.

Variable	Groups		P- Value*
	Positive Echogenic Liquor No. (%)	Negative Echogenic Liquor No. (%)	
PET			
Yes	16 (18.4)	7 (8.1)	0.047
No	71 (81.6)	80 (91.9)	
Emergency C/S			
Yes	61 (70.1)	11 (12.6)	0.001
No	26 (29.9)	76 (87.4)	
Admission To NICU			
Yes	60 (69.0)	18 (20.7)	0.001
No	27 (31.0)	69 (79.3)	
Neonatal Death			
Yes	6 (6.9)	0 (0)	0.023
No	81 (93.1)	87 (100.0)	
Apgar Score At 5 Min.	6 ± 0.9	8 ± 1.4	0.001

Discussion

In our study, we choose to include cases with a sonographically homogeneous echogenic fluid according to the criteria established by Helewa et al⁽⁸⁾. The incidence of US report of echogenic AF at term gestation during the study period was found to be 2% (100/5100) in comparison to 6.95 % (66/950) with Mungen E et al, using the same U/S criteria to diagnosis echogenic AF⁽⁹⁾. In our study, U/S echogenic liquor to be meconium–stained AF at delivery was 60.9%, vernix 29.9%, and bloody-stained AF 9.2%, in comparison to 13 (14.9%) meconium and 74 (85.1%) vernix in women with echo-free liquor detected by U/S (P= 0.001). The sensitivity and specificity of abdominal ultrasonic homogenous echogenic AF in detecting meconium-stained AF at delivery in our study were 80.3% and 68.5 % respectively with an accuracy of 73%. Helew et al⁽⁸⁾ reported the sensitivity of abdominal ultrasonic homogenous echogenic AF in detecting meconium-stained AF at delivery was 100%; specificity was 69%. In the current work, abdominal ultrasound showed a confidence of 37.9% in the diagnosis of meconium-stained AF in women with and without echogenic AF. However, echogenic liquor (test positive) detected by U/S will increase the confidence in seeing meconium–stained AF at delivery to 60.9% (PPV), with a probability of 32.5% to get a false positive test. On the other side, echo-free liquor (test negative) will rule out a possible diagnosis of meconium at delivery with a confidence level of 85.1%, A false negative test result will have a probability of 19.7 %. Helew et al. reported that the PPV was 10%; NPV was 100%; prevalence was 3.3%; and accuracy was 70%⁽⁸⁾.

According to previous research, the frequency of MSAF increased with increasing gestational age at birth. This data lends credence to the theory that MSAF may be a physiological occurrence that represents normal gastrointestinal tract maturation under the control of the fetal autonomic nervous system. When comparing echo-free AF to the group of individuals with echogenic AF, PET was statistically substantially higher ($P= 0.047$)^(10, 11). Other risk factors (maternal hypertension, DM, and hypothyroidism) were more frequent among cases compared to controls but statistically, there was no difference with the study group. E. Mungen et al. reported no statistical significance in PET between study groups (4.55%) and control group (3.62%)⁽⁹⁾.

The current study found that women with echogenic AF had a considerably greater risk of emergency cesarean sections (70.1 vs. 12.6%) than those with echo-free AF. According to Becker's study, there is a higher frequency of surgical interventions in the MSAF group when compared to the clear fluid group. Specifically, 17.4% of patients in the meconium group had C/S, whereas the control group had C/S in 9.6% of cases ($P=0.010$)⁽¹²⁾. C/S was three times more prevalent in the MSAF group, according to Naqvi⁶⁷. Wong discovered that C/S affected 13.2% of MSAF compared to 8.8% of clear AF. Navin⁷¹ reported a 49.1% C/S rate, whereas Patil reported 42%⁽¹³⁾. According to Sasikala et al., patients with MSAF require close monitoring during labor to improve perinatal outcomes, although MSAF by itself is not a sign that a cesarean surgery is necessary. However, according to E. Mungen, there was no statistically significant difference in the incidence of C/S between women with echogenic AF (31.9%) and those with echo-free U/S (33.7%)⁽⁹⁾. Even though, Abramovici et al. were unable to show a connection between the meconium's appearance and the fetal fate. Moreover, the existence of meconium did not indicate fetal compromise and did not call for medical intervention if there were no anomalies in the fetal heart rate. According to Low and Paul et al., there is a significant rise in fetal asphyxia when there is MSAF⁽¹⁴⁾. In our investigation, patients with positive echogenic AF had a significantly lower mean Apgar score at five minutes as compared to those with negative free AF. Starks had stated that their 5-minute Apgar score was much lower⁽¹⁵⁾. According to Naqvi⁽¹⁶⁾, MSAF patients had an unsatisfactory Apgar score two times more frequently. Becker, however, could not discover any statistically significant variation in the Apgar score of meconium subgroups⁽¹²⁾. The meconium's direct vasoconstrictor action on the umbilical vein, which causes vasospasm and poor placental blood flow, might be the reason for the low Apgar score. According to published research, the meconium itself may be harmful to the tissues and organs of the fetus. It causes vascular necrosis, induces constriction of the umbilical vessels, and may result in thrombi that cause tissue ischemia. Meconium, although it is sterile, decreases the amniotic fluid's antibacterial capacity by changing the zinc content, which makes intra-amniotic infection easier⁽¹⁶⁾.

According to the current study, patients with positive echogenic AF had a considerably higher incidence rate of NICU admission (69% vs. 20.7%) than the control group. A 4% MAS incidence was found in the meconium group by Naqvi. Patil recorded MAS of 12.8%. However, according to E.Mungen, there was no discernible difference in the 3.03% NICU admissions between the echogenic group and the 2.60% in the control group^(9, 13, 16).

Conclusion

The confidence of echogenic ultrasound in detecting meconium observed at membrane rupture was 37.9%, with sensitivity, specificity, and accuracy of 80.3%, 68.5%, and 73% respectively. A positive test of echogenic AF on U/S was highly significant in the prediction of cesarean section and neonatal admission to ICU because of meconium aspiration syndrome. therefore, every ultrasound showing echogenic amniotic fluid should be considered a risk factor. Training obstetrician residents to be experienced in evaluating the echogenicity of amniotic fluid in obstetrical units & labor rooms is recommended.

References

1. Ten Broek CM, Bots J, Varela-Lasheras I, Bugiani M, Galis F, Van Dongen S. Amniotic fluid deficiency and congenital abnormalities both influence fluctuating asymmetry in developing limbs of human deceased fetuses. *PLoS One*. 2013;8(11): e81824.
2. Geer LA, Pycke BF, Sherer DM, Abulafia O, Halden RU. Use of amniotic fluid for determining pregnancies at risk of preterm birth and for studying diseases of potential environmental etiology. *Environmental research*. 2015 Jan 1; 136:470-81.
3. Singh A, Mittal M. Neonatal microbiome—a brief review. *The Journal of Maternal-Fetal & Neonatal Medicine*. 2020 Nov 16;33(22):3841-8. Arnoldi R, Leva E, Macchini F, Di Cesare A, Colnaghi M, Fumagalli M, Mosca F, Torricelli M. Delayed meconium passage in very low birth weight infants. *European Journal of Pediatric Surgery*. 2011 Dec;21(06):395-8.
4. Usta, I.; Mercer, B.; Sibai, B. Risk factors for meconium aspiration syndrome. *Obstetrics and gynecology* .2005;86 (2):230-4.
5. Mashiane SE, van Dyk B, Casmod Y. Ultrasound biosafety: Knowledge and opinions of health practitioners who perform obstetric scans in South Africa. *Health SA*. 2019 Oct 17; 24:1028.
6. Magann EF, Chauhan SP, Doherty DA, Barrilleaux PS, Martin JN, Morrison JC. Predictability of intrapartum and neonatal outcomes with the amniotic fluid volume distribution: a reassessment using the amniotic fluid index, single deepest pocket, and a dye-determined amniotic fluid volume. *Am J Obstet Gynecol*. 2003 Jun;188(6):1523-7; discussion 1527-8.
7. Singh G, Archana G. Unraveling the mystery of vernix caseosa. *Indian J Dermatol*. 2008;53(2):54-60. doi: 10.4103/0019-5154.41645. PMID: 19881987; PMCID: PMC2763724.
8. Helewa M, Manning F, Harman C. Amniotic fluid particles: Are they related to a mature amniotic fluid phospholipid profile? *Obstetrical and Gynecology* 1989; 74:893-6.
9. E Mungen, Unit of Perinatology, GATA Haydarpasa Training Hospital, Uskudar, Istanbul, Turkey. *Int. Journal of Obstetrical and Gynecology*. 2005; 88:314-15.
10. Ahanya SN, Lakshmanan J, Morgan BL, Ross MG. Meconium passage in utero: mechanisms, consequences, and management. *Obstetrical and Gynecological Survey*. 2005; 60:45-56. Quiz 73-74.
11. Oyelese Y, Culin A, Ananth CV, Kaminsky LM, Vintzileos A, Smulian JC. Meconium-stained amniotic fluid across gestation and neonatal acid-base status. *Obstet Gynecol*. 2006; 108:345-9.
12. Becker S, Solomayer E, Dogan C, Wallwiener D, Fehm T. Meconium-stained amniotic fluid- perinatal outcome and obstetrical management in low-risk suburban population. *Eur J Obstet Gynecol Reprod Biol*. 2007; 123(1):46-50.
13. Patil K P, Swamy MK, Samatha K. A one-year cross-sectional study of management practices of meconium-stained amniotic fluid and perinatal outcome. *J Obstet Gynecol India*, 2006; 56(2): 12830.
14. Metti Hanoudi B, Mohammed Murad A, Duraid Ali A. Meconium staining of amniotic fluid: A clinical study. *British Journal of Medicine and Medical Research*. 2013 Oct 22;4(3):914-21.
15. Stark A R, Jane S. L, Meconium aspiration, *Manual of Neonatal Care*, 2003; 5: 402-3.
16. Naqvi SB, Manzoor S. Association of meconium-stained amniotic fluid with perinatal outcome in pregnant women of 37-42 weeks gestation. *Pak J Surg* 2011; 27(4): 292-8.