International Journal of Contemporary Pediatrics
Yadav M et al. Int J Contemp Pediatr. 2018 Nov;5(6):2309-2313
http://www.ijpediatrics.com
pISSN 2349-3283 | eISSN 2349-3291

DOI: http://dx.doi.org/10.18203/2349-3291.ijcp20184302

Original Research Article

Value of examination of the gastric aspirate for the diagnosis of neonatal infection

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Received: 21 July 2018
Accepted: 09 October 2018

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ABSTRACT

Background: Infections in early neonatal period are one of the important factors responsible for high perinatal mortality and neonatal morbidity in developing countries. This study aims to know the role of Gastric aspirates cytology in predicting the early septicemia in newborn babies.
Methods: The study will be conducted on 100 neonates with suspected septicemia and 50 normal neonates admitted to neonatology section. Gastric aspirate sample was collected for sepsis screening of neonates. All the collected data was tabulated and statistically analysed by using SPSS 2.0 software.
Results: The sensitivity of gastric aspirate cytology is 50%, specificity 65.62%, positive predictive accuracy 47.6%, negative predictive accuracy of 67. 7%. The relationship between gastric aspirate cytology and maternal risk factors was found to be statistically not significant except PIH and PROM in which association is significant. The relationship of prolonged rupture of membranes with gastric aspirate cytology was found to be highly statistically significant.
Conclusions: The chances of positivity of gastric aspirate increase as the duration of rupture of membranes increased. Gastric aspirate had high percentage of specificity and negative predictive accuracy. No specific and significant correlation between positive gastric aspirate cytology and rural/urban area, birth weight, sex, gestation, prolong labour, meconium stained amniotic fluid, mode of delivery was found.

Keywords: Cytology, Gastric aspirate, Neonate, Patiala, Sepsis

INTRODUCTION

Infections in early neonatal period are one of the important factors responsible for high perinatal mortality and neonatal morbidity in developing countries. A number of independent observers have suggested that several different laboratory determinations are individually helpful in detecting bacterial infection in the newborn infant.

Respiratory tract secretions are swallowed by the newborn even before birth, study on gastric aspirate may be more helpful for diagnosis of pulmonary infections at this age. Presence of gastric aspirate cellularity of over

75%. Polymorph nuclear leukocytosis has been suggested to indicate underlying pneumonia.[1,2] The present study has been conducted to find out the utility of gastric aspirate cytology in predicting the development of subsequent neonatal sepsis. Examination of the gastric aspirate of the newborn for cells and bacteria has been claimed to be a simple and accurate screening test for the diagnosis of septicemia.[3]

Gastric polymorphs have thus been assumed to represent a fetal intra-amniotic inflammatory response. This test is simple and can be done without specially trained staff and in a rural district hospital. This is of great importance in a developing country with limited resources and high infection rates.[4] Respiratory tract secretions are usually swallowed by the new born the study on gastric aspirates may be more helpful for the diagnosis of pulmonary infection in this age group. Examination of gastric contents is a rapid and reliable method of early diagnosis of neonatal sepsis, provided the aspiration is done within an hour of birth.

METHODS

The study will be conducted on 100 neonates with suspected septicemia and 50 normal neonates admitted to neonatology section of Department of Pediatrics, Govt. Medical College/ Rajindra Hospital, Patiala. The institutional ethical committee approved our study design. Informed consent was taken from enrolled patients attenders.

The study criteria include: Fever just before or within 7 days during or immediately after delivery, Premature rupture of membranes, Foul smelling liquor, Prolonged labour, Dai Handling, Obstructed Labour, Multiple Vaginal examination, Instrumentation during delivery, Birth Asphyxia, Respiratory distress syndrome, Intraventricular hemorrhage, Congenitally malformed babies, Small for gestation age and large for gestational age, Fever, Poor activity, refusal to feed/ decreased feeding Breathing problem, Abnormal cry Seizures Loose stools, Abdominal distention, Skin and soft tissue infection and excludes: DIC, Coomb's positive hemolytic Anemia. Difficult – resuscitation.

Methods

Collection and method of Gastric Aspirate[5]

Gastric contents were aspirated within 6 hours of birth using a sterile feeding tube and glass syringe. This time factor is important because the pH of the gastric juice is alkaline at birth and the increasing acidity of gastric secretion acts as an antiseptic and renders the result fallacious. A drop of gastric aspirate was taken on an already cleaned glass slide. A drop of heparin was added as a clearing agent and the material was spread out with the help of the flat of another glass slides. The smear thus obtained was stained. Papanicolaou staining was found to be the ideal staining procedure for the gastric aspirate smears because the cells were well stained and therefore promptly identified, the nucleus was distinct and better visualized because of the clarity of the cytoplasm, but this would have necessitated the availability of a specially trained person for staining which would have defeated the basic purpose and utility of the test and therefore it was abandoned.

Ultimately, the usual technique of Leishman staining was found to be satisfactory provided a fresh stain was made every 15-20 days. The slide was studied under high power magnification for evidence of polymorphonuclear cells. The numbers of cells per high power field were counted in a number of fields and a mean was taken. Positive gastric aspirate cytology was defined as: Gastric aspirate leucocyte count of 5 or more per high power field at the time of birth.

Data analysis

Statistical testing was conducted with the statistical package for the social science system version SPSS 17.0. Continuous variables are presented as mean ± SD, and categorical variables are presented as absolute numbers and percentage. The comparison of normally distributed continuous variables between the groups was performed using Student's t test.

RESULTS

The sensitivity of gastric aspirate cytology is 50%, specificity 65.62%, positive predictive accuracy 47.6%, negative predictive accuracy of 67. 7%. Out of total 100 cases 60 (60%) are male and 40 (40%) are female. Of 60 (60%) male, 28 (46.6%) had positive GAC of which 6 (21.42%) had proven sepsis, 15 (53.57%) probable sepsis, 7 (25%) no sepsis and 32 (53.3%) had negative GAC of which 5 (15.62%) had proven sepsis, 10 (31.25%) had proven sepsis, 17 (53.12%) no sepsis. The difference of sex of newborn with gastric aspirate cytology was not found to be statistically significant. 65 (65%) are from rural area and 35 (35%) are from urban area.

Of 65(65%) rural cases 32 (49.23%) had positive GAC of which 6 (18.75%) had proven. Of 35 (350/o) urban cases, 18(51.42%) had positive GAC of which 4(22.2%) had proven sepsis,9(50%) had probable sepsis,5(27.27%) had no sepsis. The difference of rural and urban area with gastric aspirate cytology was not found to be statistically significant. The correlation of birth weight with positive cytology is statistically not significant. 74(74%) are born term and 26(26%} are born preterm.

Of 74 (74%) cases, 32 (43.2%) had positive GAC of which 6 (18.7%) had proven sepsis, 21 (65.6%) had probable sepsis, 5 (15.6%) had no sepsis and 42 (56.7%) had negative GAC of which, 6 (14.2%) had proven sepsis, 17 (40.4%) had probable sepsis, 19 (45.23%) had

no sepsis. Of 26 (26%) preterm, 18 (69.2%) had positive GAC of which 4 (22.2%) had proven sepsis, 8 (44.4%) had probable sepsis, 6 (33.3%) no sepsis and 8 (30. 7%) had negative GAC of which 4 (50%) had proven sepsis, 2 (25%) had probable sepsis, 2 (25%) had no sepsis. The difference of gestation with gastric aspirate cytology was found to be statistically not significant.

Table 1: Incidence of sepsis by GAC with maternal risk factor.

Risk factors	GAC		Proven sepsis (n=20)	Probable sepsis (n = 48)	No sepsis (n = 32)
Cases (100)					
Prom 48 (48%)	+	22 (45.83%)	4 (18.18%)	14 (63.63%)	4 (18.18%)
	-	26 (54.16%)	3 (11.5%)	10 (38.46%)	13 (50%)
Prolong labour 10 (10%)	+	5 (50%)	1 (20%)	2 (40%)	2 (40%)
	-	5 (50%)	1 (20%)	2 (40%)	2 (40%)
MSAF 20 (20%)	+	12 (60%)	3 (25%)	8 (66.6%)	1 (8.33%)
	-	8 (40%)	4 (40%)	4 (50%)	0
PIH 22 (22%)	+	11 (50%)	2 (18.18%)	5 (45.45%)	4 (36.36%)
	-	11 (50%)	2 (18.18%)	3 (27.27%)	6 (54.54%)

Table 2: Incidence of sepsis by GAC with gestation of neonate.

Gestation		GAC	Proven sepsis (n=20)	Probable sepsis (n=48)	No Sepsis (n = 32)
Cases (100)					
Term 74 (74%)	+	32 (43.24%)	6 (18.75%)	21 (65.62%)	5 (15.62%)
	-	42 (56.75%)	6 (14.28%)	17 (40.47%)	19 (45.23%)
Pre-term 26 (26%)	+	18 (69.23%)	4 (22.2%)	8 (44.4%)	6(33.3%)
	-	8 (30.7%)	4 (50%)	2 (25%)	2 (25%)
Control (50)					
Term 38 (76%)	+	0 (0%)	-	-	-
	-	38 (100%)	-	-	38 (100%)
Pre-term 12 (24%)	+	0 (0%)	-	-	-
	-	12 (100%)	-	-	12 (100%)

Table 3: Incidence of sepsis by GAC with birth weight of neonate.

Birth weight	GAC		Proven sepsis (n=20)	Probable sepsis n=48)	No sepsis (n = 32)
Cases 100					
< 2.5 kg 36 (36%)	+	22 (61.11%)	8 (36.36%)	9 (40.9%)	5 (22.72%)
	-	14 (38.88%)	6 (42.85%)	4 (28.57%)	4 (28.57%)
> 2.5 kg 64 (64%)	+	8 (43.75%)	2 (7.14%)	20 (71.42%)	6 (21.42%)
	-	36 (56.25%)	4 (11.11%)	15 (41.66%)	17 (47.22%)
Control (50)					
< 2.5 kg 14 (28%)	+	0 (0%)	-	-	-
	-	14 (100%)	-	-	14 (100%)
> 2.5 kg 36 (72%)	+	0 (0%)	-	-	-
	-	36 (100%)	-	-	36 (100%)

The relationship between gastric aspirate cytology and maternal risk factors was found to be statistically not significant except PIH and PROM in which association is significant. The relationship of prolonged rupture of membranes with gastric aspirate cytology was found to be highly statistically significant.

DISCUSSION

Neonatal infections have been the subject of frequent discussion as they have a major impact on immediate health status of the neonate and may affect their later growth and development and may result in sequelae in

later life. Gastric aspirate fluid cytology as a quick diagnostic test, suggestive of neonatal sepsis, has been found to be a simple and reliable test.[6] Moreover it also reflects a different source of neonatal contamination. Respiratory secretions are swallowed by newborn even before birth and hence gastric fluid cytology may be more helpful in predicting pulmonary infections at this stage. However, considered presence of more than twelve cells per high power field to be indicative of infection.[7] In the present study, the presence of more than five polymorphs per high power field was considered as significant. Kumar et al used m-ESR>lOmm/I5t hr as cut off value.[8] In the present study the presence of m-ESR>lOmm at end of first hour was considered significant. In the present study gastric aspirate had sensitivity of 50% and specificity of 65.62% with positive predictive accuracy of 47.6% and negative predictive accuracy of 67. 7%, m-ESR had sensitivity of 60%, specificity 62.5%, positive predictive accuracy 50% and negative predictive accuracy of 71.5%,the combined sensitivity is 50%,specificity 81.25%,positive predictive accuracy 62.5% and negative predictive accuracy of 72.2%So the overall results in the present series are in confirmatory with those of Ebers et al and Ekwall et al.[9,10] However, the results of Aggarwal et al are less comparative with the present series as they undertook different criteria for positivity i.e. they took total number of cells whether polymorphs or lymphocytes in determining positivity as against only polymorphs considered in the present series.[4]

Various workers like Ramos et al found highly significant correlation between gastric aspirate cellularity and duration of leaking membranes.[11] In their cases with rupture of membranes with duration ranging from less than 12 hours, from 13 to 24 hours, from 25 to 48 hours and more than 48 hours, negative cytology was observed in 58.2%, 51.7%, 39.4%, 39.1% of cases respectively. However, it was interesting to note that, in one of their groups with no leaking membranes, 35.3% had positive cytology (more than five cells per high power field. In the present study, it was observed that 48 cases (48%) had rupture of membranes in their mothers and22 (45.83%) had proven sepsis.

Out of 48 (48%) cases with premature rupture of membranes, 11 cases had rupture of membranes within 0-6 hrs of which 3 (27.27%) had positive GAC of which 3 (100%) had probable sepsis, 0(0%} had no sepsis and 8 (72. 72%} had negative GAC of which 3 (37.5%) had probable sepsis, 5 (62.5%) had no sepsis (Table 1).

Eleven cases had rupture of membranes within 7-12 hours of which 3 (27.2%) had positive GAC of which 2 (66.6%) had probable sepsis, 1 (33.3%) had no sepsis and 8 (72.7%) had negative GAC of which 6 (75%) had probable sepsis, 2 (25%) had no sepsis. Seventeen cases had rupture of membranes within 13-24 hours of which 10(58.8%) had positive GAC, of which 0 (0%) had proven sepsis, 8 (80%) had probable sepsis, 2 (20%) had no sepsis and 7 (41.1%) had negative GAC of which 1 (14.5%) had proven sepsis, 1 (14.2%) had probable sepsis, 5 (71.4%) had no sepsis (Table 3).

Nine cases had rupture of membranes for>24 hours of which 6(66.6%) had positive GAC of which 4 (66.6%) had proven sepsis, 1 (16.6%) had probable sepsis, 1 (16.6%) had no sepsis and 3(33.3%) had negative GAC of which 2 (66.6%) had proven sepsis, 1 (33.3%) had no sepsis. However, the relation of gastric aspirate cytology was statistically highly significant (P value=0.009). It is therefore obvious that result of present study regarding relationship between gastric aspirate cytology and duration of leaking membrane are conformity with those of Lal et al.

It is further important to note that presence of cells in gastric aspirate is not entirely dependent upon the leaking membranes and history of leaking membranes, alone, is inadequate as a reliable method to screen babies at risk of infection. In present series a useful correlation has been observed between duration of rupture of membrane and subsequent neonatal sepsis while none of the neonates with rupture of membranes less than 24 hours duration in their mother developed infection with positive cytology, and 66.6% having rupture of membrane more than 24 hours in their mothers had positive cytology and developed infection.

In the present study, labour has been defined as prolonged when second stage of labour was more than two hours in a primigravida and more than one hour in multigravida. Mothers of 10(10%) neonate had prolonged labour. Fifty percent of neonate had positive gastric aspirate cytology and 20% developed subsequent infection. The findings of relationship of positive cytology with prolonged labour in the present study are in confirmatory with those of other workers described above. The results were however, statistically insignificant (P value = 0.947)

As regards the relationship between positive cytology and sex of the neonate, it was observed that there was preponderance of positive cytology in females (53.3%) as compared to that in the males (46.6%). It was further observed that 21.42% of males with positive cytology developed infection; the incidence of infection was more than that (15.62%) in females.

The difference was however not statistically significant (P value = 0.387). As regards the relationship between positive cytology and mode of delivery it was observed that 68. 75% cases born by caesarian section had positive cytology and 36.36% had subsequent infection as against 46.42% born by normal vaginal delivery had positive cytology and 15.3% had subsequent infection. There is high incidence of positive cytology in cases born by caesarian section. Though the difference was not statistically significant (P value=0.449). As regards the relation between positive cytology and rural and urban

area, it was observed that 49.23% cases from rural area had positive cytology and 18. 75% subsequently develop sepsis and 51.42% cases from urban area had positive cytology and 22.22% had subsequent sepsis. The difference is statistically not significant (P value=0.886). As regards the relation between positive cytology and gestation, it was observed that 43.24% of term baby had positive cytology and 18.75% had subsequent infection and 69.23% of preterm baby had positive cytology and 22.2% had subsequent infection (Table 2).

The difference though is not statistically significant (P value=0.579). The present study revealed that 20% of mothers had meconium stained amniotic fluid and of these 60% had positive cytology and 25% developed subsequent infection and 40% had negative cytology and 40% had subsequent sepsis, though the association was not statistically significant (P value=0.368). The present study revealed that 22% mothers of neonates had pregnancy induced hypertension and 50% had positive cytology and of these 18.18% developed subsequent infection and 50% had negative cytology and 18.18% had subsequent sepsis, though the association was statistically significant (P value=0.021).

CONCLUSION

The chances of positivity of gastric aspirate increase as the duration of rupture of membranes increased. Gastric aspirate had high percentage of specificity and negative predictive accuracy. No specific and significant correlation between positive gastric aspirate cytology and rural/urban area, birth weight, sex, gestation, prolong labour, meconium stained amniotic fluid, mode of delivery was found.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Yadav M, Gupta R, Singh J, Saraswath S, Nagaraj N, Bana SK. Value of examination of the gastric aspirate for the diagnosis of neonatal infection. Int J Contemp Pediatr 2018;5:2309-13.