Original Article

The role of gastric aspirates cytology and micro-erythrocyte sedimentation rate in predicting the early septicemia in newborn babies

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ABSTRACT

Background: Sepsis is an important cause of neonatal morbidity and mortality. The magnitude of problem may be reduced by early detection of amniotic fluid infections and appropriate treatment of the neonate. **Objective:** The objective of this study was to know the role of gastric aspirates cytology and micro-erythrocyte sedimentation rate (m-ESR) in predicting the early-onset septicemia in newborn babies. **Materials and Methods:** The study was conducted on 100 neonates with suspected septicemia and 50 normal neonates admitted to neonatology section of a tertiary care hospital. Blood sample and gastric aspirate sample were collected for sepsis screening of the neonates. All the collected data were tabulated and statistically analyzed using SPSS 2.0 software. **Results:** About 55% of neonates had positive cytology and m-ESR and 22.2% had subsequent sepsis. Combined sensitivity was 50%, specificity was 81.25%, positive predictive value (PPV) was 62.5%, and negative predictive value (NPV) was 72.2%. Of the 45% of cases with positive cytology and micro-ESR, 13.3% had septicemia, 6.6% had pneumonia, and 2.2% had meningitis. The other 55% of cases had negative cytology and m-ESR, and out of them, 9.09% had septicemia, 7.2% had pneumonia, and 1.8% had meningitis. The sensitivity of m-ESR was 60%, specificity was 62.5%, PPV was 50%, and NPV was 71.5%. The combined sensitivity was 50%, specificity was 81.25%, PPV was 62.5%, and NPV was 72.2%. **Conclusion:** Combined gastric aspirate and m-ESR had high percentage of specificity and NPV. No specific and significant correlation between positive gastric aspirate cytology and rural/urban area, birth weight, sex, gestation, prolong labor, meconium-stained amniotic fluid, and mode of delivery was found.

Key words: *Cytology, Gastric aspirate, Micro-erythrocyte sedimentation rate, Neonate, Sepsis*

Sepsis is an important cause of neonatal morbidity and mortality. Early diagnosis of sepsis is difficult due to its non-specific clinical presentation. Infections in early neonatal period are one of the important factors responsible for high perinatal mortality and neonatal morbidity in developing countries. Amniotic fluid infections contribute significantly to early neonatal septicemia, even with intact membranes. The magnitude of problem may be reduced by early detection of amniotic fluid infections and appropriate treatment of the neonate. A number of independent observers have suggested that several different laboratory determinations are individually helpful in detecting bacterial infection in the newborns. Due to low sensitivity and specificity of these test individually, a combination of tests was studied to evaluate a reliable septic screen, but all these studies had included one or more costly and cumbersome laboratory tests, for example, C-reactive protein, procalcitonin, or fluid culture tests. As cost is a major limiting factor in developing countries, it was felt appropriate to evolve a cheap and easy screening for neonatal septicemia.

In a situation, where neonatal infection is a common problem, the screening test would have to be simple and capable of being performed in a clinical side laboratory without specially trained personnel. Examination of gastric contents is a rapid and reliable method of the early diagnosis of neonatal sepsis, provided the aspiration is done within an hour of birth. Respiratory tract secretions are swallowed by the newborn even before birth, the study on gastric aspirate may be more helpful for the diagnosis of pulmonary infections at this age. The presence of gastric aspirate cellularity of over 75% polymorphonuclear leukocytosis has been suggested to indicate underlying pneumonia [1]. In comparison, ear canal material is influenced by drying and its examination may be difficult. These hematological parameters, which are micro-erythrocyte sedimentation rate (m-ESR), gastric aspirate cytology (GAC), and B/N ratio, can be easily done with little cost and had been given less important for the diagnosis of septicemia until in recent past [2].

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]. The present study has

been conducted to find out the utility of GAC in predicting the development of subsequent neonatal sepsis.

MATERIALS AND METHODS

The study was conducted at the Department of Pediatrics of a Government Medical College, Patiala. Total of 100 neonates with at-risk of sepsis neonates and 50 normal neonates admitted to neonatology section of the hospital. The institutional ethical committee approved our study design, and informed consent was taken from the parents or legal guardians of the enrolled patients. Neonates with following risk factors were included in the study: Maternal fever just before or within 7 days during or immediately after delivery, premature rupture of membranes (PROM), foul-smelling liquor, prolonged labor, dai handling, obstructed labor, multiple vaginal examination, instrumentation during delivery, neonates having respiratory distress syndrome, intraventricular hemorrhage, small for gestation age, fever, poor activity, refusal to feed/decreased feeding, breathing problem, abnormal cry seizures, loose stools, abdominal distention, skin, and soft tissue infections. Neonates having disseminated intravascular coagulation, Coombs-positive hemolytic anemia, and difficult resuscitation were excluded from the study.

Blood samples for m-ESR were collected by simple method of heel puncture under all aseptic precautions using a standard 75 mm heparinized microhematocrit tube with an internal diameter of 101–102 mm. One end of the tube was sealed with 2–3 mm of clay and excess of blood was wiped off from the opposite end. The tube was placed immediately in a Plexiglas rack to hold the tube vertically. The distance from the top of tube to the meniscus of the packed red cell column after 1 h was the value reported, expressed as mm/l h.

Gastric contents were aspirated within 6 h 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 which 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 and the smear obtained was stained. Although Papanicolaou staining is the ideal staining procedure for the gastric aspirate smears, it requires the availability of a specially trained person. This would have defeated the basic purpose and utility of the test, and therefore, it was abandoned.

Ultimately, the usual technique of Leishman staining was considered 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 GAC was defined as gastric aspirate leukocyte count of 5 or more per high-power field at the time of birth and subsequently 10 or more. All the demographic details of the included neonates were collected. Septic screening including complete blood counts, C-reactive proteins, and blood culture was sent for all the recruited neonates. All the collected data were tabulated and statistically analyzed using SPSS 2.0 software.

RESULTS

Our study revealed that of 100 cases, 45% had positive cytology and m-ESR and 22.2% subsequently developed sepsis which was diagnosed by sepsis screening and blood culture results. Total 55.5% of neonates with birth weight <2.5 kg had positive cytology and m-ESR while 40% developed sepsis, and 39% of neonates with birth weight >2.5 kg had positive cytology and m-ESR and 8.1% developed sepsis. In our study, 40.5% of neonates with born term had positive cytology and m-ESR and 20% developed sepsis, and 57.6% of neonates with born preterm had positive cytology and m-ESR and 26.6% developed sepsis.

Total 41.6% of cases with PROM had positive cytology and m-ESR and 20% developed sepsis, and 60% of cases with meconium-stained liquor had positive cytology and m-ESR. The other 25% developed sepsis, and 40% of cases with prolong labor had positive cytology and m-ESR and 25% developed sepsis, and 40.9% of cases with pregnancy-induced hypertension had positive cytology and m-ESR and 22.2% developed sepsis. The incidence of sepsis diagnosed by GAC in comparison to the different duration of PROM is shown in Table 1. Total 45% of cases had positive cytology and m-ESR, of which 13.3% had septicemia, 6.6% had pneumonia diagnosed by chest X-ray, 2.2% had meningitis confirmed by cerebrospinal fluid analysis, and 55% of cases had negative cytology and m-ESR, of which 9.09% had septicemia, 7.2% had pneumonia, and 1.8% had meningitis. Total 50% of cases had positive cytology and 20% had subsequent sepsis.

The present study revealed that 56% of cases had positive m-ESR and 21.4% had proven sepsis Table 2. The sensitivity of GAC was 50%, specificity was 65.62%, positive predictive value (PPV) was 47.6%, and negative predictive value (NPV) was 67.7%. The sensitivity of m-ESR was 60%, specificity was 62.5%, PPV was 50%, and NPV was 71.5%. The combined sensitivity was 50%, specificity was 81.25%, PPV was 62.5%, and NPV was 72.2%. Combined gastric aspirate and m-ESR had high percentage of specificity and NPV.

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, leading to squeal in later life. GAC as a quick diagnostic test, suggestive of neonatal sepsis, has been found to be a simple and reliable test [5]. 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 [6]. In the present study, the presence of >5 polymorphs per high-power field was considered as significant. Khudr *et al*. [7] used m-ESR >l0 mm at the end of 1st h as cutoff value and we also consider m-ESR >l0 mm at the end of 1st h as significant.

Table 1: Incidence of sepsis by GAC with the duration of rupture of the membranes

PROM	Presence	GAC (%)	Proven sepsis (%)	Probable sepsis (%)	No sepsis (%)
1 (0–6 h) (n=11)	Present	3 (27.27)	0	3 (100)	0
	Absent	8 (72.72)	0	3 (37.5)	5 (62.5)
2 (7–12h) (n=11)	Present	3 (27.27)	0	2 (66.6)	1 (33.3)
	Absent	8 (72.72)	0	6 (75)	2 (25)
3 (13–24 h) (n=17)	Present	10 (58.8)	0	8 (80)	2 (20)
	Absent	7 (41.1)	1 (14.28)	1 (14.28)	5 (71.42)
4 (>24 h) (n=9)	Present	6 (66.6)	4 (66.6)	1 (16.6)	1 (16.6)
	Absent	3 (33.3)	2 (66.6)	0	1 (33.3)

PROM: Premature rupture of membranes, GAC: Gastric aspirate cytology

Table 2: Incidence of septicemia with m-ESR

Neonates	Sepsis	m-ESR (%)	Proven sepsis n=20 (%)	Probable sepsis n=48 (%)	No sepsis n=32 (%)
Cases (100)	Present	56 (56)	12 (21.4)	32 (57.14)	12 (21.4)
	Absent	44 (44)	8 (18.18)	16 (36.36)	20 (45.45)
Control (50)	Present	0 (0)	-	-	-
	Absent	50 (100)	-	-	50 (100)

m-ESR: Micro-erythrocyte sedimentation rate

In this study, GAC had sensitivity of 50% and specificity of 65.62% with PPV of 47.6% and NPV of 67.7% while m-ESR had sensitivity of 60%, specificity 62.5%, PPV of 50%, and NPV of 71.5%. The combined sensitivity was 50%, specificity 81.25%, PPV was 62.5%, and NPV was 72.2%. The results in our study were in collaboration with those of Ebers *et al.* and Ekwall *et al*. [8,9]. However, the results of Agarwal *et al.* [4] were less comparative as they took different criteria for positivity, that is, total number of cells (whether polymorphs or lymphocytes) as against only polymorphs considered in the present series.

Various workers like Ramos *et al.* [10] found highly significant correlation between gastric aspirate cellularity and duration of leaking. In their study, in cases with leaking of <12 h, 13–24 h, 25–48 h, and >48 h, negative cytology was observed in 58.2%, 51.7%, 39.4%, and 39.1% of cases, respectively. However, it was interesting to note that in one of their groups with no leaking, 35.3% had positive cytology. In the present study, 48% of cases had PROM in their mothers and 22 (45.83%) had proven sepsis. Of 48% of cases with PROM, 11 had rupture within 0–6 h. Of 11 cases, 3 (27.27%) had positive GAC all three had probable sepsis, while 8 (72.72%) had negative GAC; of which, 3 (37.5%) had probable sepsis and 5 (62.5%) had no sepsis. 11 cases had rupture within 7–12 h; of which, 3 (27.2%) had positive GAC (two probable sepsis and one no sepsis) and 8 (72.7%) had negative GAC (six probable sepsis and two no sepsis). 17 cases had rupture within 13–24 h; of which, 10 (58.8%) had positive GAC (none with proven sepsis, eight - probable sepsis and two - no sepsis) and 7 (41.1%) had negative GAC (one - proven sepsis, one - probable sepsis, and five - no sepsis). Nine cases had leaking for >24 h; of which, 6 (66.6%) had positive GAC (four - proven sepsis, one - probable sepsis, and one - no sepsis) and 3 (33.3%) had negative GAC (two - proven sepsis and one - no sepsis).

In our study, the relation of GAC with the duration of PROM was highly significant (p=0.009) which is in conformity with those of Lal *et al*. It is further important to note that the presence of cells in gastric aspirate is not entirely dependent on the leaking and history of leaking alone is inadequate as a reliable method to screen babies at risk of infection. In the present series, a useful correlation has been observed between the duration of PROM and subsequent neonatal sepsis. While none of the neonates with PROM <24 h of duration in their mother developed infection with positive cytology, and 66.6% having PROM >24 h in their mothers had positive cytology and developed infection.

In the present study, labor has been defined as prolonged when the second stage of labor was >2 h in a primigravida and >1 h in multigravida. Mothers of 10% of the neonate had prolonged labor. 50% of the neonate had positive GAC and 20% developed subsequent infection (p=0.947). These findings of the present study are similar to those observed by other workers [6-8]. It was observed that 68.75% of cases born by cesarean 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 (p=0.449). In our study, it was observed that 43.24% of the term babies had positive cytology and 18.75% had subsequent infection while 69.23% of the preterm babies had positive cytology and 22.2% had subsequent infection (p=0.579). These findings were similar to the findings of other previous studies [5-8].

CONCLUSION

Combined gastric aspirate and micro-ESR had a high percentage of specificity and negative predictive accuracy. There is preponderance of gastric aspirate positivity in neonates of mothers with premature rupture of membranes and pregnancy-induced hypertension as risk factors. The chances of the positivity of gastric aspirate increase as the duration of rupture of the membranes increased.

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