INTRAPARTUM BACTERIOLOGY OF AMNIOTIC FLUID

A Diagnostic Aid for Imminent Maternal and Foetal Infection

By

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SUMMARY

Intrapartum Bacteriology of amniotic fluid obtained by transabdominal amniocentesis or at caesarean section was carried out in the presence of ruptured membrane in 30 patients and compared with 30 controls having intact membranes with the aim of detecting intrauterine infection before its clinical manifestation. An attempt was made to search for an early warning sign of imminent foetal and maternal infection, and to correlate various clinical parameters to its pathophysiology.

Introduction

In utero the foetus is protected by placenta, chorionic membranes, amnion and fluid but clinical and morphologic evidence proves that these occasionally fail. Consequently infections are predominant among obstetric complications. Chorio-amnionitis is an inflammatory reaction of foetal membranes. Clinically it is defined as maternal temperature more than 38°C. The pathogenesis of chorioamnionitis is not yet clear. Various hypothesis ascribed are:

(i) Ascending vaginal and cervical infections in the presence of ruptured membranes or even intact membranes.

(ii) Contamination of vagina with bowel contents.

(iii) Occasionally blood borne infections.

(vi) Aggravating factors like digital examinations, poor nutritional and socio-economic status.

Many authors believe that vaginal bacteria are present in the amniotic fluid after the onset of labour, even though the membranes are intact (Moreson, 1952; Benirschke *et al*, 1960). It was considered that once the foetal membranes are ruptured the potential for ascending infection is great.

Material and Methods

For the study thirty pregnant females in labour were randomly selected with the criterion of ruptured foetal membranes of more than six hours duration and an equal number of pregnant females with intact membranes served as control. Amniotic fluid was collected by transabdominal amniocentesis or during caesarian section under aseptic

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precautions and was transported to the laboratory immediately in anaerobic conditions.

Samples were subjected to direct microscopic examinations with gram staining and cultured aerobicaly and anaerobicaly in an atmosphere of carbon dioxide and hydrogen. Bacterial identification was done.

Observations

No obvious significant difference in the culture results among the methods used for collection of fluid was observed. Significant relationship was observed between positive culture and smears showing the presence of bacteria on gram stain. It was observed that 77.8% smear positive cases in the study group and 85.7% in the control group yielded positive culture of amniotic fluid. On the other hand the presence of white blood cells was not significantly correlated with the positive culture result (Table I).

The culture result is briefly shown in Table IIa and IIb.

TABLE I
Microscopic Examination Vs. Culture Result

Microscopic Examination	No. of Smears	Positive Culture	%	Negative Culture	%	P. value
Study group						
(a) *WBCs*						
Present	12	7	58.3	5	41.7	0.05
Absent	18	6	33.3	12	66.7	
(b) *Bacteria*						
Present	9	7	77.8	2	22.2	0.02
Absent	21	6	28.6	15	71.4	
Control group						
(a) *WBCs*						
Present	9	5	55.6	4	44.4	0.05
Absent	21	5	23.8	6	76.2	
(b) *Bacteria*						
Present	7	6	85.7	1	14.3	0.01
Absent	23	4	17.4	19	82.6	

TABLE IIa
Bacteriology of Amniotic Fluid

Group	Aerobes	Anaerobes	Mixed (Aerobes + Anaerobes)	Total culture +ve cases
Study group N = 30	10	2	1	13 (43.3%)
Control group N = 30	7	1	2	10 (33.3%)

N = Number of patients

TABLE IIb
Bacteriology of Amniotic Fluid

Species of Bacterial Isolated	Study group	Control group
Staphylococcus	5	3
—haemolytic streptococcus	1	0
Escheretia Coli	2	0
Proteus species	0	1
Clostridia	1	1
Flavobacterium	0	1
Staphyloccus + —haemolyticcus Streptococcus	1	1
Staphylococcus + Candida albicans	1	0
Proteus sp + —haemolyticcus streptococcus	0	1
Staphylococcus + Peptostreptococei	0	1
Staphylococcus + Clostridia	0	1
Clostridia + Basteriodes	1	0
Clostridia + Peptostreptococei + Proteus sp.	1	0

Bacteria were isolated in thirteen cases (43.3%) with ruptured membranes and ten (33.3%) cases with the intact membranes. Aerobic bacteria were found in the majority of cases i.e. 77% in the study group and 70% in the control group. Rest showed pure anaerobes and mixture of aerobes and anaerobes. Majority of the samples had growth of single bacterial agent. Aerobic bacteria isolated were staphylococcus, -haemolytic streptococcus, Escheretia Coli and Proteus species. Anaerobes cultured were clostridia peptostreptococcus and Bacteriodes Stapylococcus was the commonest organism isolated.

Table (III) depicts the relationship of duration of ruptured membranes at the time of collection of sample and culture result of amniotic fluid. There was apparently increase incidence of amniotic fluid infection i.e. 57% within short duration of ruptured membranes (< 12 hours) but the relationship was statistically insignificant. As duration of ruptured membranes increases there was no increase in occurrence of amniotic fluid infection (with the exception of one case).

It was observed that there was apparent increasing tendency in amniotic fluid infection with decreasing gestational age, but statistically the relationship was insignificant (Table IV). Similarly no significant increase in infection

TABLE III
Bacteriological Correlation with Duration of Rupture of Membranes

Duration of Rupture of Membranes	Number	Positive Culture	Percentage	P Value
6 - 12 hrs.	14	8	57.3	
12 - 18 hrs	12	3	25.0	
18 - 24 hrs.	3	1	33.3	>0.5
>24 hrs.	1	1	100.0	
Total	30	13	43.3	

TABLE IV
Bacteriological Correlation with Gestational Age

Gestational Age (in weeks)	Study Group			Control Group		
	No.	+ve Culture	%age	No.	+ve Culture	%age
30 - 34	1	1	100	0	0	0.0
34 - 36	4	2	50	3	2	66.6
38 - 40	7	3	43.0	4	1	25.0
36 - 38	17	7	41.0	18	5	27.0
>40	1	0	0.0	5	2	40.0
Total	30	13	43.3	30	10	33.3

rate was observed with increased number of vaginal examinations done in labour in both the groups.

Foetal Outcome (Table IV)

There was significantly better Apgar score in the uninfected control group as compared to the infected control group. In both the groups (i.e. in 60 cases) four (6.6%) neonatal deaths were observed. Three (75%) out of four deaths were in infected cases.

In the study group the main cause of neonatal death was prematurity superimposed with septicemia whereas in control group the main cause of death was septicemia probably due to intrauterine infection. The organism grown in amniotic fluid in patients with neonatal septicemia was -haemolytic streptococcus in all the three cases with a mixed growth of staphyloccus in two.

Maternal Outcome

No serious maternal complications were obseved in both the groups.

Discussion

It was universally believed by most of the obstetricians that the risk of ascending intrauterine infection is eliminated if membranes are intact and risk increases with duration of their rupture. A number of studies indicate that the bacteria can be cultured from amniotic fluid even with intact membranes. Similarly in the present series it was observed that the rupture of membranes was not associated with an increased risk of infection (i.e. 33.3% infection with intact membranes and 43.3% with ruptured membranes). Comparable results were observed by Miller *et al* (1980) in 35% and Garite *et al* (1982) in 25% positive culture with ruptured membranes. The results in the control group were supported by some studies indicating 25% (Bobbit *et al*, 1981) and 42% (Miller *et al*, 1980) of positive cultures with intact membranes. Therefore, it is suggested that the vaginal bacteria in the amniotic fluid after the onset of labour with intact membranes penetrate the mechanically intact but devitalised membranes in the area of the dilating cervix. This view is supported by similar views of other workers (Moresen, 1952; Benirschke, 1982).

The role of WBCs in amniotic fluid as an indicator of impending infection is doubtful. In the present series no significant correlation was found in both groups. Similar observations were made

in other reports (Miller *et al*, 1980; Garite *et al*, 1982 and Maye *et al*, 1983). On the other had the presence of bacterias on gram staining of smear correlated significantly with culture results (Table I). These results are in line with those of Miller *et al* (1980); Garite *et al* (1982). However, contrary to this view no relationship was observed by Bobbit (1981). Negative bacterial culture in cases with positive bacterial in the smears could be due to administration of antibiotics prior to collection of sample. The gram staining of amniotic fluid for the presence of bacteria is predictive of culture result and indirectly imminent infection, but absence of bacteria does not rule out infection.

Staphyloccus was the commonest organism isolated while Escheretia Coli recovered additionally from patients with ruptured membranes. This seems to confirm the view that intrauterine infections in the presence of ruptured membranes are caused by the gut flora like Esch. Coli and enterococcus. Therefore, the mode of infections seems to be via ascending route (H. Garson, 1973). Pomrance (1974) and Lewis *et al* (1976) observed that the incidence of amniotic fluid infection was directly related to duration of rupture of membranes but in the present series it is insignificant as also supported by Miller *et al* (1980). The majority of patients showing positive culture had premature rupture of membranes. Hence, it supports the view that premature rupture of membranes may be preceded by occult intra-amniotic infection (Ledger, 1979; Naeye and Peter, 1980).

An inverse relationship was observed between amniotic fluid infection and gestational age (Miller and Pumpkin, 1978; Miller *et al*, 1980). In the present series also an apparent increase in incidence of amniotic fluid infections has been observed but it statistically insignificant (Table V). Again no relationship was observed with number of vaginal examinations done in labour even with ruptured membranes, also observed by Miller *et al* (1980). Hence, multiple vaginal examination can be utilised to follow the progress of labour without risk of increase in infection. Neonatal outcome in the present study was more adversely affected as compared to maternal outcome. Infection was a significant contributory factor in neonatal deaths. It seems that neonatal spesis is a sequele to infection of amniotic fluid acquired during intrauterine life. Neonates born to mothers with bacteriologically proved infection of amniotic cavity developed higher incidence of clinical infection (2.1%) than in those with sterile amniotic fluid (0.2%). Alpha-haemolytic streptococcus was responsible for septicemia in neonates as also observed by Lislwa *et al* (1976), Garite *et al* (1982).

To conclude transabdominal amniocentesis is safe procedure and can be utilised for bacteriology of amniotic fluid. Significant correlation between the presence of bacteria on gram staining with culture results of amniotic fluid can be used as indirect method to predict imminent infection. Intact membranes do not contribute as an effective barrier to intrauterine infection and risk does not increase after rupture of membrane, which may be rather the result of occult intrauterine infection. No significant correlation between bacterial colonization of amniotic fluid and duration of rupture of membrane; and number of vaginal examinations could be observed. Thus later can be utilised aseptically for progress of labour in the presence of absent membranes with safety. Intrauterine infection

TABLE V
Details of Neonatal Outcome

Neonatal Morbidity or Mortality	Infected		Uninfected	
	Study group N = 13	Control group N = 10	Study group N = 17	Control group N = 20
Apgar score at Birth				
>7	10	7	14	17
7 - 4	2	1	1	3
<4	1	2	2	0
Birth Weight				
1 - 2 Kg.	2	0	2	0
2 - 3 Kg.	8	7	10	14
>3 Kg.	3	3	5	6
Neonatal Deaths	1	2	1	0
Seplicemia	2	1	0	0
Hyperbilirubinemia	0	1	0	0

N = Number of patients

was observed to have more adverse effect on neonates than mother.

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