

Investigating the Rate of Group B Streptococcus in Below 3 Months Year Old Infants with Sepsis Clinical Symptoms Hospitalized in Ghaem Hospital of Mashhad

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Abstract

Introduction

Group B streptococcus is the main reason of neonatal infection in developed countries and causes a widespread clinical indications. In developing countries such as Iran, its rate of appearance is not determined. With regard to colonization and the relative high epithelia of group B streptococcus, it is likely that the incidence of group B streptococcus in neonatal sepsis is so high. In the present study, we attempted to use the molecular methods to identify this bacterium and develop the culture environments as well.

Methods

In the present study, a hundred below three months year old infants with sepsis hospitalized in ICU sector of Ghaem hospital for one year were studied since Khordad 1388 (June 2010). After getting consent from the infants' parents, three blood samples of these patients in the sterile container with lid were transferred to the laboratory (two samples for culturing in normal environment and the other for PRC). All of the information was filled out by doctors in the incidence. SPSS 11.5 and descriptive-analytic tests were used for data analysis.

Results

With regard to the high rate of anti-biotic consumption by mothers before delivery, it is necessary to use more sensitive methods like PCR to identify the Group B Streptococcus.

Conclusion

Acceptable results and a growing survival rate was observed in this series of patients and we hope better results with improvements in minimally invasive methods.

Keywords

Sepsis, Below Three Months Year Old Infants, Group B Streptococcus, PCR(Polymeras Chain Readins).

Introduction

Group B streptococcus is the main reason of neonatal infection in developed countries and in the research conducted in Asian countries, it is known as the most prevalent factor of neonatal early sepsis (1). Bilroot described streptococcus in Erysipelas secretion and ulcer infections for the first time in 1874. In 1879, Pasteur found the same

bacterium in the blood of a patient with sepsis after the delivery (2). Many species are parasite for human and animal and create the important part of natural floor while other species create pathogens. For instance, Streptococcus Agalactia or group B Streptococcus is the most important factor of neonatal sepsis and Meningitis (3). The incidence of toxic sepsis in neonatal bacterial is changeable

and in developed countries, it is changeable in 1-4 per 1000 live infants which is different in different times and geographical regions. The male infants are infected by toxic sepsis two times more than female infants. This gender difference is determined in LBW lightweight infants. In case of existing chorioamnionitis in mother, congenital immunity disorders, Galactosamine Asplenia (*E. coli*), and malformations leading to entering the considerable amount of bacterium (obstructive uropathy), it is highly likely that the incidence of toxic sepsis in light-weighted infants increases considerably. To decrease the transference of GBS from mother to embryo and neonatal side effects of Premature Rupture of Membranes, the anti-biotic is prescribed during the delivery. In America, after using prophylaxis anti-biotic during the delivery, early neonatal infection has decreased to prevent from the transference of GBS by the time of delivery (4). As the 50 % of GBS infection transfers from infected mother to infant, only 1-2 % of infants who are colonized by this infection suffer from the early infection of group B Streptococcus. The main cause of 50 percent of neonatal early infections is Placenta and the infants get GBS infection by this way. It is necessary to know the midwifery risk factors which result in early GBS infection (5,6). In a research done for determining the incidence of B Streptococcus and the clinical indications in the north of Italy, the findings have shown that among the live births, 56 cases had attacking diseases of which 30 cases had early diseases and 26 cases had the late diseases and the incidence of group B Streptococcus infection was 0.5 per 1000 live birth. The findings have shown that 22 cases of these mothers are under the screening of group B Streptococcus vaginal infection which was positive in five patients and negative in 17 patients (7). In a ten year study in India which was done to investigate the indication of clinical symptoms and the prognosis of infants with B Streptococcus, the findings have shown that the most prevalent symptoms were Tachypnea which were indicated in infants with early infection and the results suggest that the incidence of B Streptococcus in the west of India was low (8). A study was done on 0-3 year old infants against the attacking B Streptococcus infection in Copenhagen and the toxic sepsis of attacking B Streptococcus infection was indicated which its incidence was 0.76 per 1000 live birth. The total result of this study has indicated early GBS infection reduction in

Denmark (22). In a study in America, the results have shown B Streptococcus infection increase in pregnant women (from the birth to 7 days) and the late infection remained stable (7-90 days) and increasing the incidence of early infection is related to the black race. In researches in Iran, the rate of vaginal colonization of group B Streptococcus was reported to be 9 -27 % in last days of pregnancy and the rate of transference to the infants was estimated 60 % (9). In the studies by Zamanzas, Rabie, Jahromi and Mosibi which were about the identification of the B Streptococcus vectors in pregnant women referring to Maternity hospitals, the results have shown the positive culture in pregnant women's vagina with regard to group B Streptococcus (9-13). The investigations by Rabie, Jahromi and Mosibi and Sarafrazi have approved the role of positive colonization in the vagina of pregnant women which cause early neonatal infection (9,11-13). Another study in Tehran which investigated the infants with sepsis and Meningitis in Mofid children's hospital for two years, has indicated that 11 % of blood cultures was positive that the most prevalent cultured organisms included Staphylococcal Apydrmatysh (47%), Staphylococcal Aureus Pseudomonas and E.coli (each 10.5 %). None of the case of B Streptococcus was reported (14). The researches on the causes of sepsis infection of B Streptococcus were conducted insignificantly. With regard to the high rate of vaginal colonization of group B Streptococcus, it is likely that the incidence of group B Streptococcus in neonatal sepsis is so high and as this bacterium causes bacteremia before 3 months of pregnancy, it is likely that the culture techniques and microbiology methods are not enough to identify the bacterium. So we attempted to use the precise and fast methods to identify the bacterium (PCR) and develop the culture environment to determine the existence of this bacterium in case of killing it and its inhibition for some reasons like taking antibiotic by pregnant women and acquire the proper techniques for pregnant women with regard to the treatment of vaginal infection and doing tests with regard to the vaginal infection and also use more effective therapeutic method against the infection of group B Streptococcus.

Methods

This study is a cohort temporary investigation. According to the previous studies by Neger, the rate of B Streptococcus infection in infants with

the sepsis clinical symptoms is reported to be 0.35 % , 96 samples were determined for this study and for this reason , 100 below 3 months year old infants were studied from Khordad 1388 (Jun 2010) to Khordad 1389 (Jun 2011). (16). All of the patients with congenital anomalies, like the Cyanotic heart disease, Neural tube defects like MMC Anencephaly, Gastrointestinal anomalies like Diaphragm hernia, kidney anomalies, and Urinary tract like Renal agenesis and etc. were excluded from the study. Also all of the hospitalized sepsis cases (cause the sepsis symptoms after 48 hospitalizing) were omitted from the study. After getting the consent from the parents, 2cc of blood were extracted for normal and developed culture environment and 0.5 cc of blood for PCR. The culture environment includes Brain Heart infusion Broth with supplement. In this environment, poly etyosolphanat Sodium (SPS) was added up during the creation of the environment to inhibit 0.025 g/l Blood coagulation. After adding the blood, the culture environment was transferred to the laboratory and was kept in Incubator under 37 centigrade degree to grow and identify the given bacterium. After 24 and 48 hours, the culture environments were investigated with regard to group B Streptococcus. In case of finding documents based on group B Streptococcus, the existence of given bacterium were approved by proper biochemistry tests. For standardizing and ensuring the quality of tests, standard operating procedure document was considered in all of the stages. One cc of another blood was kept in fridge and frozen under -20 centigrade degree after transferring to laboratory. During the test, the samples were under PCR to find B Streptococcus and firstly the bacterium was under DNA extraction by Genef bio to extract DNA. And to find the DNA of B streptococcus by a pair of Specific primers, DNA amplification was done and then the existence of given Nucleic acid were evaluated by Electrophoresis to identify B streptococcus. To conduct the PCR and determine the B streptococcus, all types of DNA in blood samples were broken up by Prime Prep TM and then B streptococcus were identified by Diagnostic tests.

Results

In this study, the newborn infants and the 90 days old infants were hospitalized in Ghaem hospital in Mashad from Khordad 1388 (Jun 2010) to Khordad 1389 (June 2011) for the reason of sepsis

clinical symptoms. The normal blood culture and the developed blood culture were extracted from the infants with regard to the environment of culture and 0.5cc of blood were set aside for PCR to investigate the B streptococcus. The findings have shown that the weight of 19 hospitalized infants was less than 2500 g at the time of birth, 35 infants between 1500 and 2500g and 42 infants more than 2500 g. Apgar score was less than 5 in 4 patients, in 16 patients between 5 and 7, and in 78 infants more than 8 at the time of birth. The age and gender distribution of infection symptoms in infants was shown in Table 1 and the clinical indications in infants were shown in graph 1.

Table1: Age and Gender Distribution of Infection Symptoms Indication in Below 3 Months Year Old Infants

Infants' ender	Infants' age,y (0-7 days old)	Infants' age,y (30 days old)	Infants' age,y (30-90 days old)
male	36	5	5
female	41	7	6
total	77	12	11

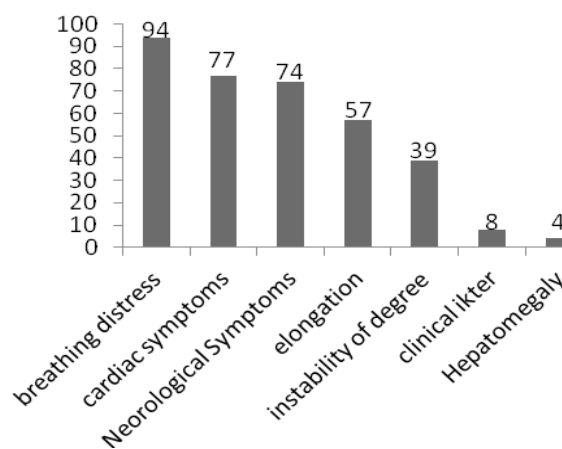


Fig 1: Clinical Indications in Below Three Months Year Old Hospitalized Infants.

The findings have shown that 12 % of infants had less than a hundred thousand Platelets and 23 % had more than 18000 White corpuscle. Regarding the ABG of patients, 63 cases had Respiratory acidosis with more than 60 PCO₂ and 21 cases had metabolic acidosis with less than 15 HCO₃. Nine patients of 36 ones, who were asked CRP, had positive CPR. The findings have shown that the normal cultures in infants resulted in inclusion of

two cases of Klebsiella, one case of Enterobacter, two cases of Aklay and one case of Streptococcus Alpha hemolytic. Three infants that were positive Polymerase chain reaction for B streptococcus had normal and developed negative culture. Fifty five of 100 infants were born by Caesarean delivery and 41 infants by natural method of delivery. Four infants were referred from the nursery. All of the Caesarean cases and nine mothers who had natural method of delivery used to take anti biotic. Nine cases of these infants died of which 5 cases had positive normal and developed culture and 6 cases had negative normal and developed culture. Three infants had positive PCR for group B Streptococcus which all of them resulted from the second delivery and their mother took anti biotic. The first case didn't show a 15 hour Rupture of Membranes and the second and third didn't show the Rupture of Membranes.

Discussion

With regard to the investigations, this study is the first one in Iran which used the molecular method to identify the group B Streptococcus in sepsis infants and newborn babies and the blood sample of three patients of 100 below three year old infants with sepsis became positive by PCR method regarding the B Streptococcus. In other countries, the identification of group B Streptococcus is done based on the bacterium culture and the molecular method has been used to identify this bacterium (17-20). In Kong and Jordon studies, PCR multiplex is used to identify the serotypes of group B Streptococcus (36, 37). In Golden research which was conducted on the sensitivity and the characteristics of real time PCR, the sensitivity and the characteristics were 100 percent and all of the 26 bacterium of non-group B streptococcus had negative PCR (19).

The results of culture by means of developed culture environment have shown the inclusion of two cases of Klebsiella, two cases of Klebsiella two cases of coli, one case of Enterobacter, and one case of Streptococcus Alpha hemolytic and any case of group B Streptococcus were not acquired. Based on the results of bacteria culture in our study, the negative bacterium consists of 83 % of positive cases which was compatible with Mosibi's findings. Twenty nine findings of Movahedian have shown that the positive organisms consist of positive cultures in contrast to our studies. In the studies by Sarafrazi and Jahromi, which was about the positive culture of group B Streptococcus,

the mothers had the positive vaginal culture and in the studies by Sarafrazi, only two cases and in the study of Jahromi, about 60 % of the infants of these mothers had positive group B Streptococcus culture and just one of the infants had group B Streptococcus sepsis (2,25). The studies by Dang in China have shown that among 200 died infants due to pneumonia, the PCR of 52 cases were positive for group B Streptococcus (20). The studies by Zamanzad, Sarafrazi, Jahromi and Rabie have shown that the pregnant women are colonize by group B Streptococcus (9-12). In other countries, group B Streptococcus is the major reason of neonatal sepsis. In a study in America which was about the group B Streptococcus infection, the results suggested that group B Streptococcus infection was the factor of infection, disease and death in infants in America(4). The present study suggested that indication of clinical symptoms in hospitalized infants included the respiratory symptoms, cardiac symptoms and neurological symptoms which were compatible with the findings of Anderson respectively. The studies of Anderson in Copenhagen have shown that incidence of Streptococcus infection was 0.76 % per 1000 live births. Thirty two studies of Truthman in Italy have suggested that Streptococcus infection was 0.5 per 1000 live births (31). Alseyed in Qatar indicated that group B Streptococcus is the most prevalent cause of Meningitis in below 3 months year old infants (17). In the present study, the results of PCR tests suggest group B Streptococcus, but why the cultures for group B Streptococcus became negative necessitates the widespread researches. The following issues are proposed to answer this question:

1. The excessive use of anti -biotic in pregnant women especially at the last days of pregnancy inhibits the growth of B Streptococcus. In our study 64 % of pregnant women have taken anti biotic before delivery.
2. The normal diet which the pregnant had at last days of pregnancy contains antibiotic materials and inhibits the growth of B Streptococcus.
3. The growth of other pathogens inhibits the growth of B Streptococcus.
4. Not using the developed systems of blood culture like Bactec containing absorbent anti biotic

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References:

1. Tiskumara R, Fakharee SH, Liu CQ, Nuntnarumit P, Lui KM, Hammoud M, et al. Neonatal infections in Asia. *Arch Dis Child Fetal Neonatal Ed.* 2009 Mar;94(2):F144-8. Epub 2008 Sep 19.
2. Infection in newborn babies available at: <http://www.aboutkidshealth.ca/Pregnancy/Infection-in-Newborn-Babies.aspx/articleID=7680&categoryID=PG-nh4-10g>. Accessed Apr 18, 2010.
3. Daley AJ, Isaacs D. Ten-year study on the effect of intrapartum antibiotic prophylaxis on early onset group B streptococcal and *Escherichia coli* neonatal sepsis in Australasia. *Pediatr Infect Dis J.* 2004 Jul;23(7):630-4.
4. Trends in perinatal group B streptococcal disease. 2009. MMWR. Available at: <http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5805a2.htm>. Accessed Jul 25, 2010.
5. Shet A, Ferrieri P. Neonatal and maternal group B streptococcal infections: a comprehensive review. *Indian J Med Res* 2004; 141-150.
6. Elsaid MF, Flamerzi AA, Bessisso MS, Elshafie SS. Acute bacterial meningitis in Qatar. *Saudi Med J.* 2006 Feb;27(2):198-204.
7. Trotman H, Bell Y. Neonatal group B streptococcal infection at the University Hospital of the West Indies, Jamaica: a 10-year experience. *Ann Trop Paediatr.* 2006 Mar; 26(1):53-7.
8. Robinson TD, Kumar P, Cadichon BS. Sepsis in the emergency department. *CPEM* 2008; 9:160-8.
9. Namavar Jahromi B, Poorarian S, Poorbarfehee S. The prevalence and adverse effects of group B streptococcal colonization during pregnancy. *Arch Iran Med.* 2008 Nov; 11(6):654-7.
10. Zamanzad B. Prevalence of vaginal strep B carrier in pregnant women attending the hospital's maternity ward Shahre cord city. *Journal of Shaeed Sdoughi University of Medical Sciences Yazd* 2002, Jun; 10(3): 27-31.
11. Sarafrazi N, Mesdaghi nia E, Moniri R, Mosavi GA. Prevalence of group B beta-hemolytic *Streptococcus* of vaginal flora in pregnancy and its relation to early neonatal infections and infections during pregnancy. *Feyz* 2001 Feb;4(18):22-7.
12. Rabiee S, Arab M, Yousefi Mashouf R. Epidemiologic Pattern of Vaginal colonization by group B streptococcus in pregnant women in Hamadan, central west of Iran. *Iran J Med Sci* 2006 Jun; 31(2): 106-108.
13. Mosayebi Z, Movahedian AH, Moniri R. Profile of bacterial sepsis in neonates from Kashan in Iran. *J Infect Dis Antimicrob Agents* 2003; 20:97-102.
14. Amid MH. Cases of sepsis and meningitis in newborns hospitalized in Mofid Children's Hospital. *Pejoughesh dar Pezeshki* 2002; 26 (57): 1-63.
15. Fatehi I, Nourozy Z, Naseri M. Review of 82 cases of meningitis in infants under the 2 months during 14-year. *TUMJ* 1998;3(1):46-51.
16. Gharebaghi MM, Maamuri GA, Peirovifar A, Boskabadi H, Afshari JT. Immediate diagnosis of early onset in premature newborns by measurement of cold C-Reactive protein and interleukin-6. *IJMS* 2007;132:217-21.
17. Kong F, Ma L, Gilbert GL. Simultaneous detection and serotype identification of *Streptococcus agalactiae* using multiplex PCR and reverse line blot hybridization. *J Med Microbiol.* 2005 Dec;54(Pt 12):1133-8.
18. Jordan JA, Durso MB. Real-Time Polymerase Chain Reaction for Detecting Bacterial DNA Directly from Blood of Neonates Being Evaluated for Sepsis. *J Mol Diagn.* 2005 November; 7(5): 575-81
19. Golden SM, Stamilio DM, Faux BM, dela Cruz WP, Shoemaker CT, Blackmon CL, et al. Evaluation of a real-time fluorescent PCR assay for rapid detection of Group B *Streptococci* in neonatal blood. *Diagn Microbiol Infect Dis.* 2004 Sep;50(1):7-13.
20. Deng JH, Yao KH, Hu HL, Yu SJ, Gao W, Fu LB, et al. Detection of group B streptococcus in the cases died of neonatal pneumonia. *Zhonghua Er Ke Za Zhi.* 2006 Nov;44(11):850-4.
21. Movahedian AH, Moniri R, Mosayebi Z. Bacterial culture of neonatal sepsis. *Iranian J Publ Health* 2006; 35:84-9.
22. Andersen J, Christensen R, Hertel J. Clinical features and epidemiology of septicaemia and meningitis in neonates due to *Streptococcus agalactiae* in Copenhagen County, Denmark: a 10 year survey from 1992 to 2001. *Acta Paediatr.* 2004 Oct; 93(10):1334-9.