THE ROLE OF PCR AMPLIFICATION OF 16S RRNA IN EARLY DIAGNOSIS OF NEONATAL SEPSIS

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ABSTRACT

Neonatal sepsis is a major cause of death in newborns despite sophisticated neonatal intensive care. This cross-section study was done on 69 neonates with suspected sepsis who were admitted in Neonatal Intensive Care Unit (NICU) of Maternity and Child Teaching Hospital at AL-Diwaniya city, and 20 healthy neonates as a (control group) in the period from March to October 2009.This study was conducted to evaluate the diagnostic value of polymerase chain reaction (PCR) for bacterial DNA component encoding 16S rRNA in the early diagnosis of neonatal sepsis prior to the blood culture (the golden standard test). The investigation protocol included blood culture and 1 mL of venous blood for molecular analysis by polymerase chain reaction (PCR) for bacterial DNA component encoding 16 s RNA in all cases, We compared the results of PCR with blood culture. The culture positivity rate 20 (28.9%) among suspected sepsis neonates, with sensitivity of (29%)and specificity of(100%).The male more affected than female among proven sepsis with ratio 3:2. It was found a high sensitivity (100%), high specificity (87.5%) and positive predictive value of (98.6), negative predictive value of (100%)for PCR analysis for bacterial DNA component encoding 16S rRNA .Blood culture is the most reliable method for diagnosis of neonatal sepsis. Polymerase chain reaction is useful and superior to blood culture for early diagnosis of sepsis in neonates.

INTRODUCTION

Neonatal sepsis is invasive bacterial infection occurring during the first month of life. The incidence of culture- proven sepsis is approximately 2 per 1000 live birth and out of the 7-13% of neonates who are evaluated for neonatal sepsis, only 3-8% have culture proven sepsis. The early signs of sepsis in the newborn are non specific and include diminished spontaneous activity, less vigorous sucking, apnea, bradycardia, temperature instability, respiratory distress, vomiting, diarrhea, abdominal distension, seizure and jaundice. Therefore, many newborns undergo diagnostic studies and the initiation of treatment before the presence of sepsis has been proven, because the mortality rate of untreated sepsis can be as high as 50% **(1)** particularly in preterm, low-birth-weight infants **(2)**. Rapid diagnosis of neonatal sepsis is problematic because the first sings of this disease may be minimal and are similar to those of various non infectious processes. Furthermore bacterial cultures are time consuming and other laboratory tests which are used lack sensitivity or specificity. In this situation neonates with risk of infection or clinical suspicion are empirically treated with antibiotics, and may lead to unnecessary increased antibiotic consumption, a higher incidence of side-effects due to their use, increased resistance to antibiotics, a long hospitalization, and separation of infants from their mothers and increased health costs**(3)**. Recently, polymerase chain reaction (PCR) based assays have been seen as having the potential to provide an early and accurate diagnosis of diseases caused by bacterial pathogens and have improved the rate of microbial detection.

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The sequences of 16S rRNA gene has been used to diagnose and identify bacterial infection in clinical practice **(4)**. Some PCR-based assays can be used to identify specific bacterial pathogens **(5)**, while broad-range bacterial PCR can detect almost any bacterial species **(6).**The use of broad-range bacterial PCR has a great advantage: it can detect microorganisms that are found less frequently or even unknown causative agents of bacterial origin **(7).**

PATIENTS & METHODS:

This cross –section study performed on 69 neonates who were admitted to Maternity and Childhood Teaching Hospital at neonatal intensive care unit (NICU) at AL-Diwaniya city, and 20 healthy neonates as a (control group) in the period from March to October 2009. They were evaluated for neonatal sepsis with blood culture and subjected to broad-rang PCR amplification for bacterial 16-S RNA gene. A total of 2ml. of blood was obtained from two different sit of selected neonates with suspected septicemia by venipuncture under sterile condition, each 1ml. of blood inoculated immediately into 60 ml. of brain –heart infusion (BHI) broth contain 0.05% sodium polyanethol sulphonate.The rubber diaphragm of the culture bottle was likewise scrubbed with povidone-iodine solution prior to needle insertion. The cultures for aerobic bacteria were incubated at 37^{o}C for 7 days under aerobic conditions, and inspected visually every morning. Subcultures were performed 24 hours after inoculation, the second on third day and a final on the seventh day. Samples were removed from each culture bottle aseptically with a sterile 1 ml syringe, and one drop was subcultured onto 5% blood agar, Chocolate agar and MacConkey agar plates, which were then incubated at 37^{o}C for 24 hours. One blood agar plate, along with the Chocolate agar, was incubated in 5%CO **2 (8).** Extraction of the DNA from blood sample by using DNA Purification Kit for Blood (Wizard. Genomic, Promega,USA).Presence of bacterial DNA in blood samples was evaluated by amplifying the DNA region encoding 16 rRNA (861 base pair) using the following primers: F-5AGAGTTTGAT-CCTGGCTCAG-3' and R-5'GGACTACCAGGGTACTTAAT-3'. The amplification used an initial modifying denaturation step (03 min at 94ºC and 03 min at 55ºC for 3 times) followed by 30 cycles (1 min at 95ºC, 1 min at 54ºC and 1 min at 72º C). A final extension cycle of 72ºC for 07min was carried out. Then detection of PCR amplified 16S r RNA gene by horizontal agarose gel electrophoresis. Statistical Analysis by using the performance characteristics (validity) of a test or criteria, include among others : sensitivity, specificity, positive predictive value and negative predictive value .

RESULTS

In this cross-section study, a total of 89 neonates included in, out of which 69 neonates were with suspected sepsis and 20 healthy neonates (control group). Among suspected sepsis neonates, 20(28.9%) neonates were with positive blood culture (proven sepsis group) and 49(71.1%) neonates were with negative blood culture (probable sepsis group). The males tend to be more affected (60%) when compared to the females (40%), with 3:2 ratio. But this is not significant with respect to the culture positivity, Table (1). The PCR test shown an important statistical significant P value <0.001,with positivity (100%), as all neonates with proven sepsis had positive 16SrDNA gene in their blood specimen which detected by PCR, also there was additional six neonates among culture negative sepsis express a positive 16SrDNA gene in their blood specimen ,Table(2). In Figure (1), Lane M: DNA Molecular weight marker(100 bp, Promega, USA), lane 1-5 : positive DNA amplified from blood-EDTA samples were taken from suspected sepsis neonates, lane 6-9: negative result from blood-EDTA samples, lane 10: negative

control . A high sensitivity (100%), high specificity (87.5%) and PPV of (98.6),NPV(100%) for PCR test. Table(3).

Discussion

Bacterial neonatal sepsis continues to be the major cause of morbidity and mortality in the newborn particularly those who are premature (8) and its prognosis largely depends on early identification and prompt treatment which are critical to ensure optimal outcome (9). Blood culture is the gold standard for the confirmation of sepsis which requires a minimum of 48–72 hours, and yields a positive result in only 30–70% of cases (10). In advanced centers, blood culture is positive in 80% of genuine sepsis. However, isolation rates vary from (6.7%) to (55.4%) (11). In present study, the rate of proven sepsis was (28.9%) which is lower than that observed by other studies in which the isolating rate was (40.3%)(12),but comparable with studies which conducted that the positivity rate were (21.5%)(13). The varying results may be due to different study population different defining of proven sepsis and as the culture positivity depend on time of sampling and the extent of bacteremia in neonate(14). In present study, sex distribution among neonates with sepsis was (60%) males and (40%) females, Table (3). These results confirm other studies which have shown that males have been reported to be 2-6 times more likely than females to develop sepsis (15). Nearly 3:2 ratio in this study is constant with this data. This male preponderance has been attributed to the deficiency of an X linked immuno-regulatory gene ,as previously mentioned in the review of literature. A predominance of male infant is apparent in almost all studies of sepsis in newborn (16). In present study, Presence of bacterial DNA in blood samples was evaluated by amplifying the DNA region encoding 16SrRNA (861 base pair) using the following primers: F-5AGAGTTTGAT-CCTGGCTCAG-3'and R-,GGACTACCAGGGTACTT AAT-3'. The PCR results yielded the sensitivity of (100%) and specificity of (87.5%), Table (3).Also the PCR has shown (100%) correlation with microbiological methods in all infected cases ,as all cases with positive blood culture yielded 861 base pair bands and among cases with negative blood culture six cases resulted in amplification of 861 base pair bands, table (2) and Figure(1). Present result similar to the a study done on 100 newborn infants at risk for EOS and found the PCR with sensitivity of (100%) and specificity of (95.6%), and the PCR was positive in 4 additional cases with positive sepsis screen but with sterile blood cultures, and concluded that the PCR is a useful tool because of the short time required to reach confirmation(17). Another study conducted on 48 neonates has found that the diagnosis of bacterial sepsis in the newborn by PCR shown (66.7%) sensitivity, (87.5%) specificity, (95.4%) positive and (75%) negative predictive value. The pathogenic bacterium was detected in the blood culture of only (19.4%) of these patients, with the molecular method of broad-range 16S rDNA PCR, the detection of bacteria improved to (29.0%). As the total number of patients was low this difference did not obtain statistical significance (18) .

PCR does not replace culture, this technique, when performed by a single skilled operator is rapid and reproducible and the clinical significance of the result is supported by the analysis of species detected (19).

CONCLUSION

The 16SrRNA PCR is a valuable molecular tool to aid in the detection of non-culturable aetiological agents.

RECOMMENDATION

It will be important, however, to continue surveillance of neonatal sepsis in order to follow closely changes in trends and risk factors, to obtain information for empirical

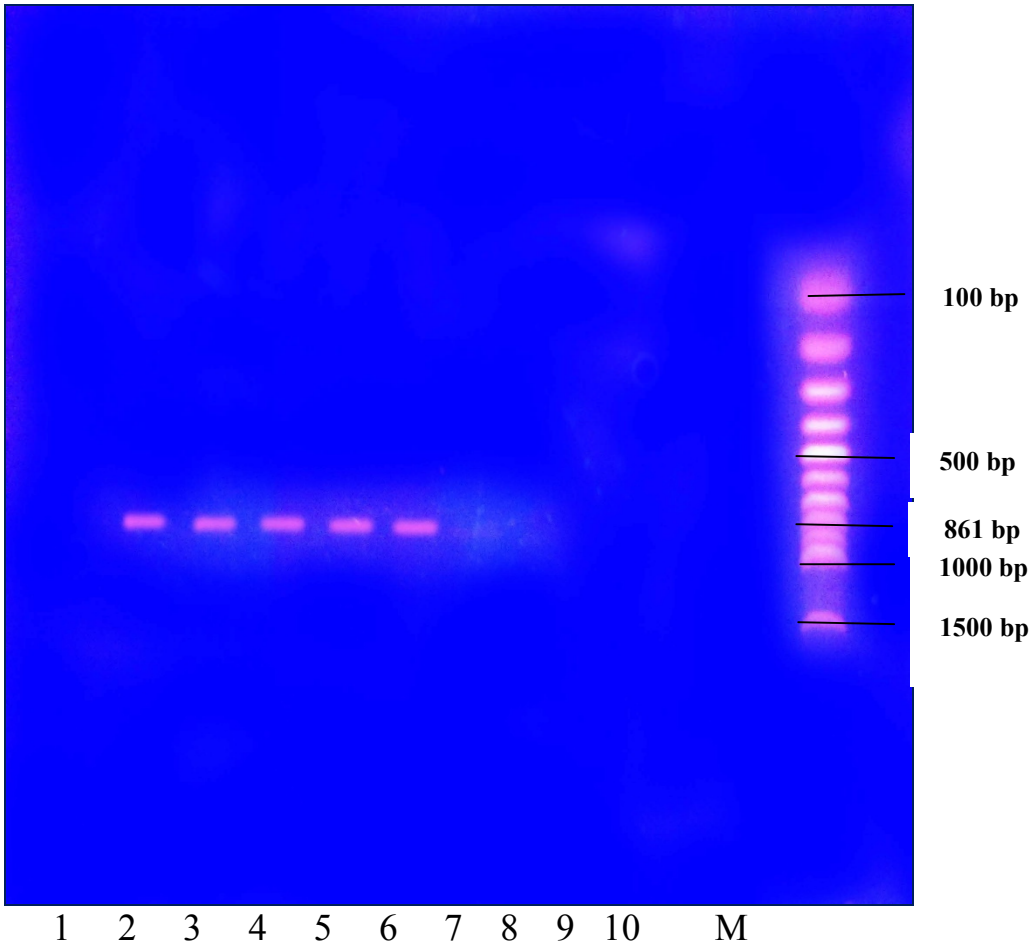
antibiotic therapy and to react rapidly to case of major changes in susceptibility patterns and Introduce modern technique such as PCR our hospitals for early diagnosis of neonatal sepsis, based on molecular detection of causative agents.

Table (1): The Difference in Gender Distribution by Study Group.

	Proven sepsis (Culture positive)		Probable sepsis (culture negative)		Healthy controls		P (Chi-square)
Gender	**NO.**	**%**	**NO.**	**%**	**NO.**	**%**	
Female	**8**	**40**	**17**	**34.7**	**6**	**30**	**0.8[NS]**
Male	**12**	**60**	**32**	**65.3**	**14**	**70**	
Total	**20**	**100**	**49**	**100**	**20**	**100**	

Table(2):The Positivity Rate of PCR Test by Study Group.

Positive Tests	Culture positive neonatal Sepsis (n=20)		Probable sepsis (culture negative) (n=49)		Healthy Controls (n=20)			Culture Positive x Culture negative cases	Culture Positive x Healthy controls
	NO	%	NO	%	NO	%			
PCR test	**20**	**100**	**6**	**12.2**	**0**	**0**	**P** **95% CI for OR**	**<0.001** **(2.15 - 8.74)**	**<0.001** **



Figure(1):The Agrose Gel Electropheresis After PCR Amplification of 16SrRNA Gene From Blood Specimen of Neonates with Sepsis.

Table(14):Validity Parameters for PCR Test When Used to Predict Culture Positive Sepsis Cases Differentiating Them from Clinically Suspected Sepsis Cases.

Positive if ≥ cut-off value	Sensitivity	Specificity	Accuracy	PPV ate pretest probability =		NPV ate pretest probability = 10%
				50%	90%	
PCR test	100.0	87.5	91.2	88.9	98.6	100.0

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دور البي سي ار في التشخيص المبكر لخمج الطفال الرضع

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الخلاصة

أجريت دراسة مقطعية على ٦٩ طفل حديث الولادة والذين تظهر عليهم علامات الأنتان الدموي من بين الراقدين في وحدة العناية المركزة في مستشفى الأطفال والنسائية التعليمي بمدينة الديوانية ، و٢٠ طفل حديثي الولادة من الأصحاء كمجموعة تحكم وللفترة من آذار حتى تشرين الأول ٢٠٠٩ ،أجريت هذه الدراسة لتقييم القيمة التشخيصية للتحليل الجزيئي عن طريق تفاعل البلمرة المتسلسل (الاسترداد) لمكون الحمض النووي البكتيري ذي الترميز ١٦ (16S rRNA gene) في التشخيص المبكر عن الأنتان الدموي عند حديثي الولادة. أظهرت الدراسة انخفاض معدل المزرعة الايجابية ٢٠ (٢٨,٩%) بين حديثي الولادة المشكوك بهم الأنتان الدموي ، والذكور أكثر تأثرا من الإناث بين المصابين بالأنتان الدموي بنسبة ٣:٢. وقد تبين وجود حساسية عالية (١٠٠%) ، خصوصية عالية (٨٧,٥%) وPPV (٩٨,٦%) ، وNPV (١٠٠%) لتحليل الحمض النووي لمكون الاسترداد البكتيري ذي الترميز ١٦ في التمييز بين حالات الإنتان المؤكدة و حالات الإنتان المشكوك فيه.

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