EARLY DIAGNOSIS OF NEONATAL BACTERIAL SEPSIS USING 16s rRNA BY POLYMERASE CHAIN REACTION

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LIST OF ABBREVIATIONS

Abbreviation	Description
AFIS	Amniotic Fluid Infection Syndrome
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
C B C	Complete blood count
C R P	C-reactive Protein
C.N.S	Central nervous system
C3	Complement 3
c3a	activated complement product
CMV	Cytomegalovirus
CONS	Coagulase - negative staphylococci
CSF	Cerebro- Spinal Fluid
CSI	Clinically suspected infection
CT	Computed tomography
DIC	Disseminated intravascular coagulopathy
DNA	Deoxyribonucleic Acid
e. g	example gradient
EOS	Early onset sepsis
ESR	Erythrocyte sedimentation rate
FDA	Food and Drug Administration
GBS	Group B Streptococci
G-CSF	Granulocyte Colony Stimulating Factor
GIT	Gastrointestinal tract
GM-CSF	Granulocyte macrophage Colony Stimulating Factor
Hb	Hemoglobin
HIV	Human immune deficiency virus
I /T	Immature-to-Total
I\M	Immature \ mature neutrophil ratio
IAP	intrapartum antibiotics prophylaxis
ICAM-1	Intracellular adhesion molecule -1
Ig	Immunoglobulin
IL-2r	Soluble interleukin-2 receptor
IL-6	Interleukin - 6
IL-8	Interleukin - 8
IUGR	Intrauterine growth retardation
IV IG	Intravenous immunoglobulin
LBW	Low birth weight

LIST OF ABBREVIATIONS (Cont...)

Abbreviation	Description
LOS	Late onset sepsis
LPS-A	Lipopolysacharide-A
MODS	Multiple organ dysfunction syndrome
MPV	Mean platelet volume
mRNA	Messenger Ribonucleic acid
NEC	Necrotizing enterocolities
NICU	Neonatal intensive care unit
PCR	Polymerase chain reaction
PCT	Procalcitonin
PDW	Platelet distribution width
PGE1	ProstaglandinsE1
PMNs	Polymorphnuclear leucocytes
PROM	Premature rupture of membrane
PT	Prothrombin Time
PTT	Partial thromboplastin time
RBCs	Red blood cells
RDS	Respiratory distress syndrome
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
rTFPI	Recombinant tissue factor pathway inhibitor
S.D	Standard deviation
SIRS	Systemic inflammatory response syndrome
TFPI	Tissue factor pathway inhibitor
TNF- a	Tumor necrosis factor - alpha
TPN	Total parenteral nutrition
tRNA	Transfer RNA
UTI	Urinary tract infection
WBCs	White blood cells

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INTRODUCTION

Infections including neonatal sepsis are still an important cause of neonatal morbidity and mortality despite development of broad spectrum antibiotics and technical advances in life support therapy. Factors which contribute to the high susceptibility of neonates to infection include; prematurity (influences of very low birth weight and also immune immaturity), maternal genital colonization, transplacental spread following maternal infection, traumatic delivery, invasive procedures such as arterial or umbilical catheterization and underlying problems such as heart disease or hyaline membrane disease ***(Hodge et al., 1998).***

Early diagnosis and treatment are crucial to improvement in prognosis of neonatal sepsis. Clinical findings are usually nonspecific and indistinguishable from those caused by a variety of neonatal non-infective disorders. Therefore, sensitive and specific markers are needed that reliably identify truly infected neonates at earliest stage of the disease ***(Kuster et al., 2001 and Ehrlich, 2002).***

Laboratory indicators, such as complete blood cell count, ratio of immature to total neutrophils and C-reactive protein do not have high sensitivity, especially if measured early in the course of sepsis. Currently, blood culture is considered the gold standard for diagnosis of bacterial sepsis ***(Kuster et al., 2001and Ruppenthal et al., 2005).***

The common approach is to start antimicrobial therapy to all infants with clinical or laboratory signs of blood infection as well as to infants with high risk of early onset sepsis. However, the results of blood culture are not available before 48-72 hours. Therefore, faster and more specific tests are required to avoid the inappropriate use of antibiotics which have been implicated in the development of multiresistant bacterial strains in hospitals ***(Terrone et al., 1999 and Ruppenthal et al., 2005).***

Molecular techniques such as polymerase chain reaction (PCR) have been used successfully to identify a wide range of organisms, including bacteria, yeasts, viruses, and protozoa. Unlike culture, these types of assays do not require growth of an organism for detection. This technology has proven to be quite useful in diagnosing non culturable pathogens like human parvovirus B19 or human papillomavirus and has the potential for excellent sensitivity. Recently, bacterial DNA consensus sequences, e.g., the 16s rRNA gene, have been identified to define an organism as a bacterium. With such sequence information available, numerous DNA primers and probes have been described for use in PCR based assays to diagnose bacterial sepsis ***(Jordan and Durso, 2005).***

The 16s rRNA (ribosomal RNA) when translated is the small subunit of the ribosome in Prokaryotes. The majority of research on the microbial populations of biomining microorganisms has focused on the analysis of the 16s rRNA gene. Determining the 16s rRNA gene of a microorganism has

become a routine part of phenotypic characterization and has replaced many culture based techniques. As the ribosome is essential to cellular function the gene remains highly conserved and can be used to determine the evolutionary relatedness of microorganisms ***(Amann et al., 2008).***

AIM OF THE WORK

The aim of the present work is to study 16s rRNA using PCR as a rapid and early marker for diagnosis of neonatal bacterial sepsis. This may help to decrease the use of antibiotics and total neonatal intensive care unit length of stay.

NEONATAL SEPSIS

Definition

The terms neonatal sepsis, sepsis neonatorum and neonatal septicemia have been used to describe the systemic response to infection in newborn infants. Previously, it was considered to involve mainly bacterial infections. However, it is now known that a great variety of microbes other than bacteria may be responsible ***(Gomella et al., 2010)*.**

Certain definitions or terms are used to describe patients with various manifestations of sepsis, **(a) Infection:** Microbial phenomenon characterized by an inflammatory response to the presence of microorganisms (bacteria, viruses, parasites, fungi) or the invasion of normally sterile host tissue by other organisms; **(b) Bacteremia:** The presence of viable bacteria in the blood; **(c) Systemic inflammatory response syndrome (SIRS):** The systemic response to a variety of severe clinical insults (infection, trauma, burns). The response is manifested by two or more of the following: temperature >38°C or <36°C, heart rate >2 standard deviations above normal for age, respiratory rate >2 standard deviations above normal for age, leucocytic count > 21.000 cells/mm3, < 4000 cells/mm3, or 10% band forms; **(d) Sepsis (SIRS plus infection):** The systemic response to infection, **(e) Severe sepsis:** Sepsis associated with organ dysfunction, hypoperfusion or hypertension; **(f) Septic Shock:** Sepsis associated with hypotension despite adequate fluid resuscitation in