INTRODUCTION

Severe infections remain important causes of neonatal morbidity and mortality ***(Qazi and Stoll, 2009).*** Early clinical features of infection are often subtle, non-specific and difficult to recognize ***(Chiesa et al., 2004).*** The usefulness of conventional hematological tests in assisting frontline neonatologists to differentiate between infected and non-infected neonates is limited ***(Ohlin et al., 2010).***

The "gold standard" for the neonatal diagnosis of sepsis is a positive microbiological culture. This method has two main limitations: firstly, the results are not available until 48 hours after blood sampling, and secondly, 57% of all fetuses with bacterial infection yield a negative culture result ***(Yoon et al., 2003).*** Therefore, the diagnosis of neonatal sepsis remains a clinical and laboratory challenge because of the non-specific symptoms as well as the low sensitivity and specificity of laboratory tests in the neonatal period of life. In clinical practice, several markers, such as C-reactive protein (CRP), white blood cell differential count, absolute neutrophil count, immature to total neutrophil ratio and microbiological tests, as well as the clinical status and symptoms, contribute to the final diagnosis ***(Levy et al., 2003).***

New readily measurable circulating biomarkers have been described as an additional tool for severity classification of septic patients and prediction of mortality in critically ill patients. Adrenomedullin (ADM) is included among these new biomarkers ***(Michels et al., 2011).***

ADM is mainly released from endothelial cells, acts as a potent vasodilator and has natriuretic effects ***(Kaplan et al., 2011).*** Other ADM properties include a reduction in endothelial permeability ***(Temmesfeld-Wollbruck et al., 2007),*** bactericidal effects and down-regulation of pro-inflammatory cytokines ***(Allakeret al., 2006).*** Thus, it is not surprising that serum ADM levels are increased in sepsis ***(Marino et al., 2014).***

The reliable measurement of ADM is challenging since it is rapidly cleared from the circulation ***(Kato et al., 2003).*** Pro-ADM (pro-ADM), a more stable precursor molecule to ADM, was reported to correlate well with other markers such as IL-6 and CRP as a predictor of prognosis in patients with sepsis ***(Cotesta et al.,2005).*** Elevations of pro-ADM have been reported in systemic inflammatory response syndrome (SIRS), sepsis, and septic shock in adults ***(Angeletti et al., 2013).***

AIM OF THE WORK

The aim of this study is to evaluate the value of pro-ADM measurement in the diagnosis of neonatal sepsis as well as, to investigate if there is any correlation between it and CRP.

NEONATAL SEPSIS

Definition

Neonatal septicemia is a clinical syndrome of systemic illness accompanied by bacteraemia occurring in the 1st month of life **(Noor et al., 2008).** It encompasses various. systemic infections of the newborn such as septicemia, .meningitis, pneumonia, arthritis, .osteomyelitis .and urinary tract infections. Neonatal infections, which may be caused by bacteria, viruses, or fungi, occur as early or late. infections and their timing gives care providers. clues for determining causative agents **(Venkatesh et al., 2006).**

Epidemiology

The rate of sepsis in infants born at any hospital varies according to the prenatal risk factors in the community of women who deliver there. Economic standards, availability of prenatal care, geographic variations, and outbreaks of cases caused by particular species of pathogen may each play a role in determining the overall rate ***(Adams-Chapman and Stoll, 2006).***

Socioeconomic factors appear to be important in determining which infants are at risk of infection. Premature infants and infants with low birth weight are more frequently

born to mothers of low socioeconomic class than to those of average or high socioeconomic class. The bacterial cause of neonatal sepsis and meningitis varies from one geographic area to another. Although the bacterial causes of neonatal sepsis in countries of Western Europe are similar to those in the United States, a different pattern has been noted in other countries such as Saudi Arabia, Nigeria and Mexico. In these countries, gram negative enteric bacilli are the predominant organisms causing neonatal sepsis and meningitis ***(van den Hoogen et al., 2009).***

Region	Neonatal Mortality	1990	1995	2000	2005	2009	Percent Change 1990–2009 (Annual)
Global	NMR	33.2	32.0	29.4	26.3	23.9	−28.0 (−1.7)
	Deaths in 1,000s (% of global)	4,574 (100)	4,296 (100)	3,913 (100)	3,536 (100)	3,265 (100)	−28.6
HIC	NMR	6.0	4.9	4.2	3.9	3.6	−40.3 (−2.7)
	Deaths in 1,000s (% of global)	79 (1.7)	63 (1.5)	52 (1.3)	48 (1.4)	45 (1.4)	−42.7
Africa (LMIC)	NMR	43.6	43.0	41.1	38.3	35.9	−17.6 (−1.0)
	Deaths in 1,000s (% of global)	971 (21.2)	1,047 (24.4)	1,094 (27.9)	1,119 (31.6)	1,114 (34.1)	14.7
Americas (LMIC)	NMR	22.0	19.0	15.8	13.1	11.4	−48.3 (−3.4)
	Deaths in 1,000s (% of global)	255 (5.6)	220 (5.1)	181 (4.6)	145 (4.1)	121 (3.7)	−52.7
Eastern Mediterranean (LMIC)	NMR	41.3	39.1	36.3	33.2	31.1	−24.6 (−1.5)
	Deaths in 1,000s (% of global)	565 (12.4)	530 (12.3)	506 (12.9)	490 (13.9)	481 (14.7)	−14.9
Europe (LMIC)	NMR	21.0	20.1	17.0	13.2	10.7	−49.2 (−3.5)
	Deaths in 1,000s (% of global)	148 (3.2)	118 (2.8)	90 (2.3)	72 (2.0)	60 (1.8)	−59.6
South-East Asia (LMIC)	NMR	46.9	43.6	39.2	34.2	30.7	−34.6 (−2.2)
	Deaths in 1,000s (% of global)	1,860 (40.7)	1,738 (40.5)	1,549 (39.6)	1,326 (37.5)	1,161 (35.6)	−37.6
Western Pacific (LMIC)	NMR	22.9	22.1	18.6	14.6	12.0	−47.5 (−3.3)
	Deaths in 1,000s (% of global)	694 (15.2)	580 (13.5)	442 (11.3)	336 (9.5)	283 (8.7)	−59.3

doi:10.1371/journal.pmed.1001080.t001

Figure (1): Percentage changes of neonatal mortality among different geographical locations from 1990-2009 (Mikkel et al., 2011).

Incidence:

Over all incidence of neonatal sepsis ranged from 2 to 20 per 1000 live births with cases fatality rates of 1 to 69%, the incidence of culture proven sepsis in the united states is approximately 2 in 1000 live births of the 7-13% of the neonates who were evaluated for neonatal sepsis, only 3-8% had culture proven sepsis. The early signs of sepsis in the newborn are non specific, therefore, many newborns undergo diagnostic studies and the initiation of treatment before the diagnosis has been determined ***(Zaidi et al., 2012).***

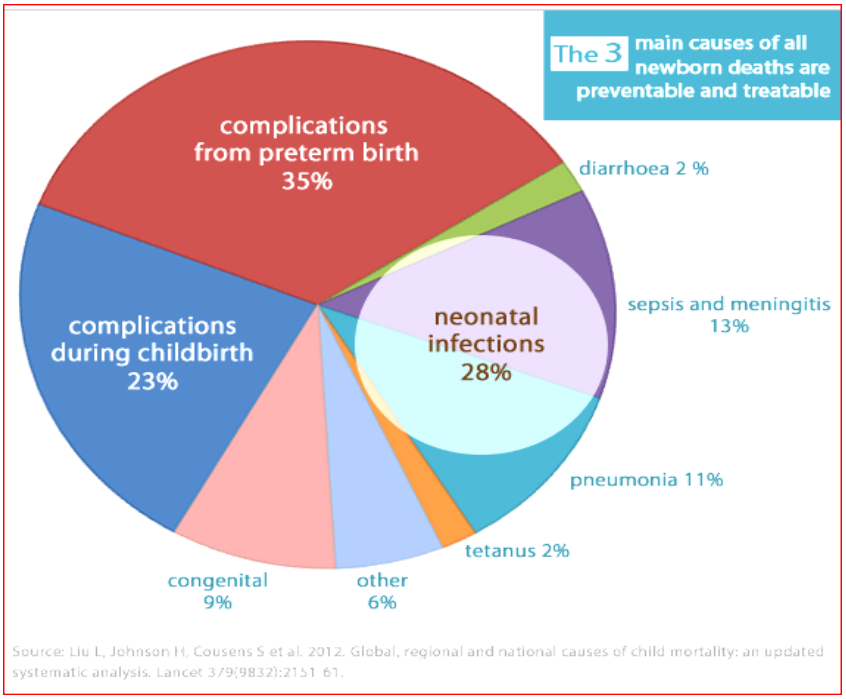

Figure (2**):** Percentages of causes of newborn mortality globally **(Liu and Johnson et al,2012)**

The World Health Organization (WHO) estimates that each year 4 million newborns worldwide die during the neonatal period. Seventy-five percent of these deaths occur during the first week of life **(Lawn et al., 2005),** and 25% to 45% of neonatal deaths occur the first day of life **(WHO 2006; Lancet et al., 2008).**

Neonatal deaths are mainly caused by severe infections (36%), prematurity (28%) and birth defects (7%) (Lawn et al., 2005).In the United States, 1 to 5 of every 1000 live births result in neonatal infection. Neonates with certain risk factors are at higher risk for developing infection/sepsis . Causative agents in neonatal sepsis are listed. The Centres for Disease Control and Prevention estimates that, for every141 babies born in the United States each year, 1 dies of infection in the first year of life. That comes to 30,000 newborn deaths, including 20,000 in the first month of life. Before the use of antibiotics, mortality rates for newborns with infection/sepsis were 95% to 100%; after use of antibiotics, mortality rates range from 13% to 45% **(Venkatesh et al., 2006).** Lower mortality rates are the result of earlier case-finding, timely diagnostic evaluation, and initiation of empiric antibiotic therapy. Both inpatient and outpatient neonatal care requires timely diagnosis and therapy; delay is associated with worsening morbidity and mortality **(Gardner et al., 2008).**

Classification:

Neonatal infections are usually classified according to time and mode of transmission. They are grouped into three categories:

1- Congenital infection, acquired in-utero by vertical transmission with onset before birth.
2- Early-onset neonatal infections, acquired by vertical transmission in the prenatal period, either shortly before or during the process of birth.
3- Late and late-onset neonatal infections acquired by horizontal transmission in the nursery. This is shown in table (2) ***(Arnon and Litmanovitz, 2008).***

Table (1): Relationship of time of onset of neonatal infection and mode of transmission of infection.

Characteristics	Prenatal	**Early onset**	**Late onset**	**Late, Late onset**
Age at onset	Prior of birth	birth to 7days usually <72hr	7 to 30 days	> 30 days
Maternal/obstetric complication	Common	Common	Uncommon	Varies
Prematurity	Maternal infections, usually primary infection prolonged rupture of membranes	Frequent	Varies	Usual
Organism source	Transplacental or ascending	Maternal genital tract	Maternal genital tract/ environment	Environment/ community
Manifestation	Multi-system	Multi-system	Multi-system or focal	Multi-system or focal
Site	Intra-Uterine	Normal nursery, NICU community	NICU, community	NICU, community

(Nelson 2004) (Altunhan et al., 2011)

Early onset sepsis:

Early onset sepsis is usually a fulminating illness often complicated by meningitis or pneumonia. Eighty-five percent of newborns with early onset infection present within 24 hours, 5% present at 24-48 hours, and a smaller percentage of patients present between 48 hours and 6 days of life ***(Lin et al., 2011).***

Onset of infection is more rapid in premature neonates, and prolonged rupture of membranes. It also has been reported that a significant proportion of the babies born to mothers with severe preeclampsia are neutropenic and more usually vulnerable to early onset sepsis ***(Volpe et al., 2008).***

Early onset sepsis syndrome is associated with acquisition of microorganism from the mother. Transplacental infection or an ascending infection from the cervix may be caused by microorganisms that colonize in the mother's genitourinary tract. The infant may acquire the microbe by passage through a colonized birth canal at delivery ***(Adams-Chapman and Stoll, 2006).***

The microorganisms most commonly associated with early onset infection include group B streptococcus(GBS), Escherichia coli, Haemophilus influenzae, and Listeria monocytogenes ***(Chan et al., 2009).***

Late onset sepsis:

Late onset sepsis syndrome occurs at 7-30 days of life and is acquired from the surrounding environment. The spectrum of infection ranges from minor skin infection to life threatening septicaemia. The infant's skin, respiratory tract, conjunctiva, gastrointestinal tract and umbilicus may become colonized from the environment. Vectors of such colonization may include vascular or urinary catheters, other indwelling lines, or contact from care givers with bacterial colonization, the mortality rate form late onset sepsis was 9% as opposed to 15% for early onset sepsis, and numerous studies have reported a much higher mortality rate from early onset sepsis ***(Enomoto et al., 2009).***

Table (2): Current predominant pathogens in early and late onset.

Early-onset:	***Late-onset:***
□□Group B streptococcus □□E. coli. □□Listeria Monocytogenes □□Staphylococcus aureus □□Other streptococci □□Other gram negative organisms: - Hemophilus influenza - Klebsiella pneumoniae - Pseudomonas aeruginosa - Enterobacter species	□□Coagulase-negative staphylococci □□Klebsiella pneumoniae □□Pseudomonas aeruginosa □□Other gram negative enteric bacteria □□Candida species

(Hoffman and Harris, 2002)

Pathogenesis:

The neonatal infections are unique due to a number of factors:

(1) There are diverse modes of transmission of infectious agents from mother to fetus or newborn infant as trans-haematogenous spread, Vertical transmission of infection in-utero, and exposure to infectious diseases in the nursery or in the community

(2) The newborn infant maybe less capable of responding to infection owing to one or more immunologic deficiencies

(3) Co-existing diseases of the newborn often complicate the diagnosis and management of neonatal infections for example; acidosis impairs the function of polymorph nuclear leukocytes

(4) The manifestations of infectious diseases in the newborn infant are extremely variable ***(Bang et al., 2005).***

(5) Transient bacteremia may accompany procedures that traumatize mucosal membranes as endotracheal suctioning, the bacteremia may also occur by direct extension from colonized mucosal surfaces ***(van den Hoogen et al., 2009).***

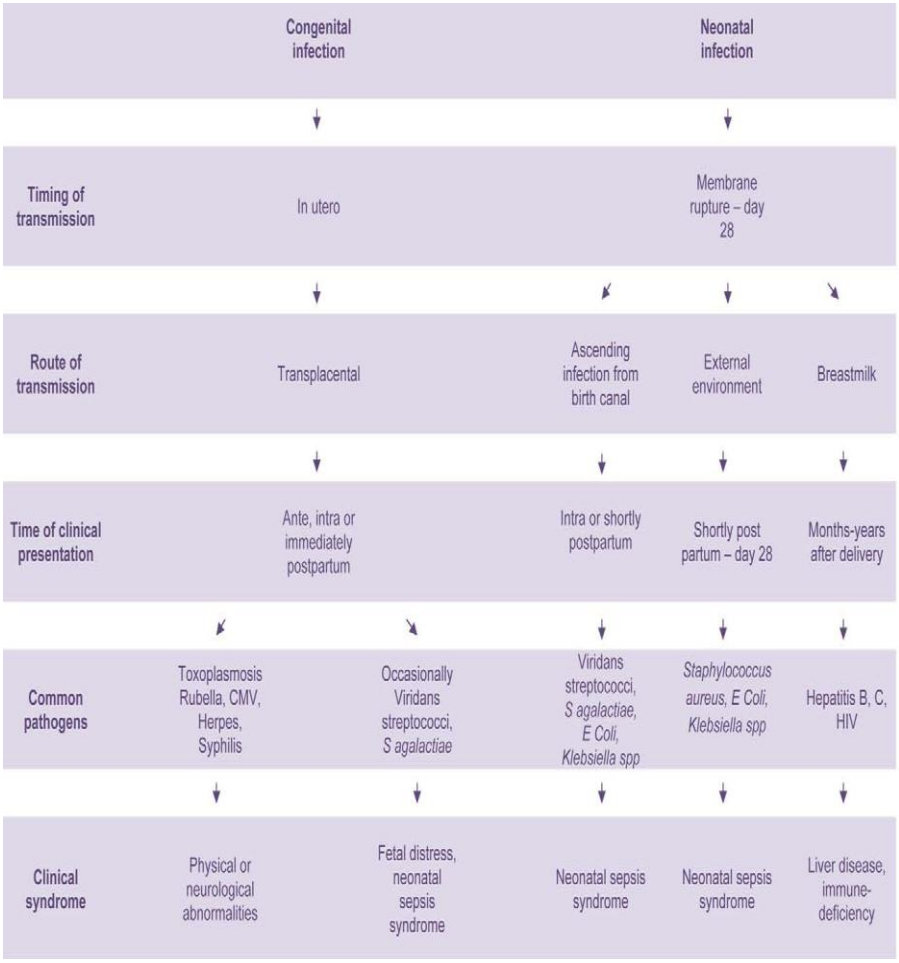

Figure (3): Pathogenesis of congenital and neonatal infection **(Edemand and Zaidi, 2010).**

Aetiology:

Both maternal, neonatal and environmental risk factors determine which infants exposed to potentially pathogenic organisms will develop sepsis, meningitis or other serious invasive infections ***(Avery, 2006).***

(A) Maternal Risk Factors:

Premature rupture of membrane especially with prematurity is a very important factor associated with sepsis. Excessive bleeding from placenta previa or abruption placenta, excessive manipulation, a prolonged second stage of labor and fetal distress during delivery are also factors that have been associated with an increased incidence of neonatal infections ***(Bang et al., 2005).***

The amniotic infection syndrome refers to bacterial invasion of amniotic fluid, usually as a result of prolonged rupture of the chorioamniotic membrane. On occasion, amniotic infection occurs with apparently intact membranes. Exposure to and aspiration of bacteria in amniotic fluid lead to congenital pneumonia or systemic bacterial infection with manifestations becoming apparent prior to delivery fetal distress, tachycardia), at delivery (prenatal asphyxia), or after a latent period of a few hours (respiratory distress, shock) ***(Lahra and Jeffery,2004).***

The infection in the female tract (especially in the cervix) can cause premature rupture of membranes and induce premature labor, this process is responsible for many preventable infant deaths. Maternal causal factors in preterm birth with its complications include short cervix and funnelled cervix which are more likely to be associated with preterm birth when the woman also has a bacterial vaginosis and/or Chlamydia infection. Fetal or placental factors such as multi-fetal gestation, and polyhydramnios which increase risk for infection ***(Goldstein et al., 2005)*.**

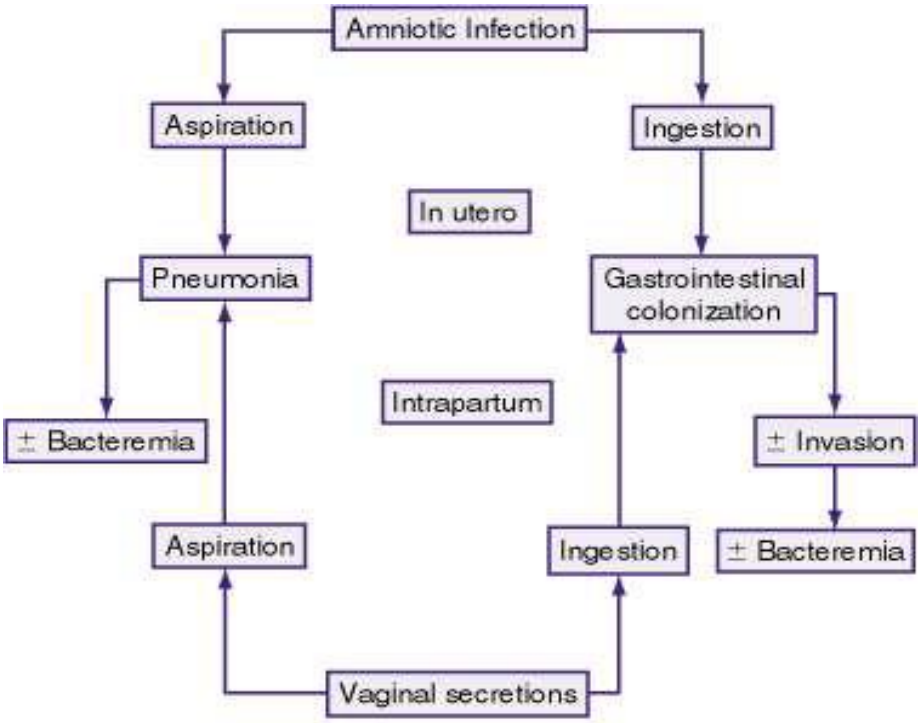

Figure (4): Pathways of ascending or intrapartum infection ***(Stoll, 2008).***

Transplacental acquisition of bacteria during the course of maternal bacteremia is difficult to establish, but it has been documented in patients infected with Listeria monocytogenes and Treponema palladium ***(Chan et al., 2009)***

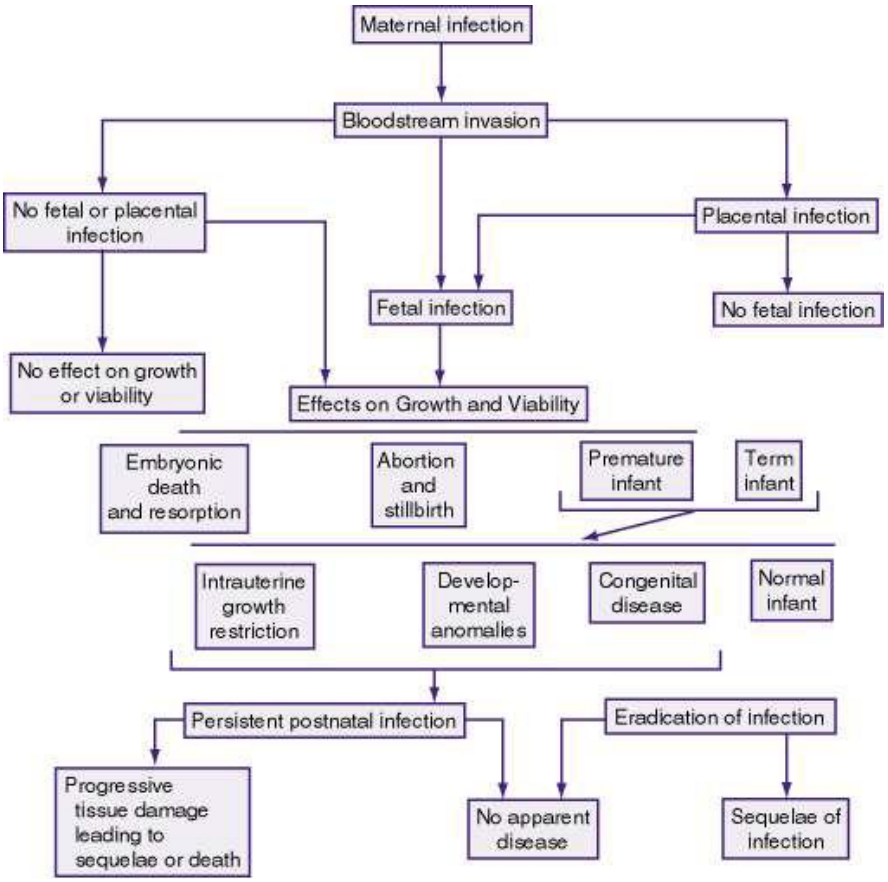

Figure (5): Pathogenesis of hematogenous transplacental infections ***(Remington and Klein, 2002).***