IMMUNE STATUS IN PRETERM BABIES AND ITS ASSOCIATION WITH SEPSIS

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ABSTRACT

Preterm delivery is the most important risk factor for both mortality and morbidity due to infections. The risk of neonatal sepsis is 4-10 times higher in low birth weight infants than in full term neonates and varies inversely with gestational age.

A cross sectional pilot study using convenient sampling method was conducted to evaluate various immunological parameters in preterm newborns, comparing them with term newborns and correlating them with the presence of neonatal sepsis. Cord blood from 36 preterm babies delivered at HUSM was analyzed. Cord blood from 36 term babies was also analyzed as control. For neutrophil function, we used Nitroblue tetrazolium (NBT) kit. For complement levels and immunoglobulin levels, we performed immunoturbidometry techniques. Lymphocyte subset analysis was performed by using tritest reagent and flowcytometric analysis.

In both preterm and term groups, the numbers of boys were more than girls, majority of the babies were born via spontaneous vaginal deliveries and most of them were breastfed. The period of amenorrhoea (POA) of mothers of preterm babies ranges from 29 to 36 weeks and the mean POA was 34.47 weeks of gestation. In term babies, POA was between 37 to 41 weeks of gestation and the mean was 38.78 weeks. The mean weight of preterm babies was significantly

lower than term babies. The gravidity of mothers of preterm babies ranges from gravida 1 to 9 whereas for term babies from gravida 1 to gravida 10.

NBT was significantly reduced in preterm babies compared to term babies (7.5% versus 12.0%). Complement levels, C3 (0.5114 versus 0.7192g/l) and C4 (0.07 versus 0.14g/l) were significantly lower in preterm babies than in the term control group. Level of C3 was comparable with previous study but the level of C4 was lower, which may be due to different method performed. When we compared level of IgG in preterm and term babies, we found that the mean IgG level in preterm babies was lower than in term babies (9.5583 versus 14.2806 g/l). The IgG level of preterm babies in this study agreed with the previous study. In the study, IgM (0.1 versus 0.2g/l) and IgA (0.210 versus 0.225g/l) levels were lower in preterm than in term babies and the levels were significantly different between these two groups.

The study has shown a number of differences between lymphocyte subsets of preterm and term babies. Only CD3% was significantly different between two groups (65.2917% versus 70.3153%). The percentages of CD4, CD8, CD 19 (B cell) and CD 16/56 (NK cell) were not significantly different.

In the study, follow-up of the patients for two weeks after birth showed that sepsis occur in 11.1% of preterm babies. However, none of term babies developed

sepsis. We also found that only NBT reduction and IgA level were associated with occurrence of sepsis in preterm babies. IgG level, IgM level, complement levels and lymphocyte subsets were not associated with occurrence of sepsis. However, this may be due to small numbers of babies.

For association of immunological parameters and other variables including gravidity, sex, method of delivery, type of feeding or birth weight, we have found that NBT reduction, IgG, IgM, C3 and C4 levels were found to be associated with birth weight whereas CD3%, CD4% and CD 16/56 (NK cell) % were associated with gravidity.

In conclusion, in this study we found that NBT reduction, IgG level, IgA level, IgM level, C3 level, C4 level and CD3% were significantly different between preterm and term babies. We also found that only NBT reduction and IgA level were associated with occurrence of sepsis in preterm babies. Other than that NBT reduction, IgG, IgM, C3 and C4 levels were found to be associated with birth weight whereas CD3%, CD4% and CD 16/56 (NK cell) % were associated with gravidity.

1. INTRODUCTION

1.1 BACKGROUND

Preterm babies tend to be very vulnerable to many complications during the early weeks and months of their lives. One of the most frequent complications preterm babies face is neonatal sepsis.

It is generally believed that the high incidence of neonatal sepsis is closely related to the state of immunodeficiency that is present in these preterm babies. Several studies have shown that different parts of the immune system tend to be less well developed in preterm neonates than in term neonates.

Very few studies however have looked at many parameters at the same time in a cohort of preterm babies and compared these parameters with each other. Very few studies have also correlated the finding of these parameters to the neonatal incidence of sepsis. Most studies done measuring and comparing the

immunological parameters in preterm and term babies date from decades back under the range of preterm infants and the limits of viability were very different from now. Furthermore no local data or reference values for these immunological parameters are available for the Malaysian neonates.

The incidence of neonatal sepsis is still high in most developing countries. A relatively high incidence of neonatal sepsis makes assessment of the importance of individual risk factors a bit easier.

This study was undertaken to evaluate a wide range of immunological parameters in preterm newborns, compare them with each other, compare them with the values found in Malaysian term newborns and correlate them with the presence of neonatal sepsis. This pilot study is an initial evaluation to identify trends and to determine the direction of a future study with a similar aim.

The introduction of this study will further cover a brief overview on the definition, epidemiology and complications of preterm babies. Special attention will be given to the incidence, diagnosis and causes of neonatal sepsis before a thorough literature review of studies looking at the immune status of preterm babies.

1.2 PRETERM BABIES

1.2.1 DEFINITION

According to the World Health Organization (WHO) recommendation (Bristol, 1972), a preterm baby is defined as baby born from 22 weeks till 36 completed weeks (less than 259 days) from the first day of the last menstrual period and the term 'premature' should no longer be used. Preterm was previously defined as a birth weight of less than 2500 g. Therefore, this includes infants of low birth weight due to early delivery or due to growth retardation but exclude preterm infants that had birth weights of more than 2500 g such as in diabetes mellitus. The problems of the preterm infants are more closely associated with chronological maturity rather than birth weight per se and it is, therefore, more meaningful to adopt the WHO nomenclature of preterm labour. Nevertheless, adoption of the term preterm labour is not without practical problems in pregnancies where the date of the last menstrual period is in doubt or unknown. Infants weighing less than 2500 g are, therefore, designated as low birth weight (LBW) babies.

1.2.2.1 INCIDENCE OF PRETERM DELIVERIES

The true incidence of preterm births varies from country to country and from one geographic region to another within a country. In 1969, the incidence of preterm births in the United States was 9.8% of all pregnancies (Monthly Vital Statistic Report, United States Department of Health, Education and Welfare, 1973). In Britain, 6.4% of all births were preterm in 1970, compared to 3.4% of all births in East Germany in 1974 (Wynn *et al*, 1977)

The rate of preterm birth has been increasing slightly in the past few years. An epidemiologic study of induced labour in the United States from 1989 to 1998 found a doubling of the induction rate for preterm pregnancies, from 6.7% to 13.4%. Occurring primarily among non-Hispanic white women with singleton births, preterm birth inductions accounted for a significant portion of the growth in the preterm birth rate during that time (Mac Dorman et al, 2002). In 2000 about 8% of babies were born preterm in Ontario (Maternal, newborn & Early Child Developmental Center, 2002).

The incidence of preterm labour and delivery increased in women who have previously had preterm births or delivery of low birth weight infants (Federick and Anderson, 1976).

The etiologic factors that have been frequently implicated in preterm labour can be classified into four major categories: maternal, fetoplacental, uterine and iatrogenic (table 1). The majority of cases of preterm labour is without apparent or detectable cause and is often referred to as idiopathic preterm labour (Yusoff, 1980).

Acute systemic illness in the mother with pyrexia, such as in pyelonephritis and febrile viral infections, can cause preterm labour and the increased uterine activity has been attributed to the prostaglandin release during pyrexia. Severe chronic systemic illnesses, such as chronic renal and cardiac diseases or chronic hypertension, are known to be associated with low birth weight infants but not necessarily preterm labour (Yusoff, 1980).

Maternal endocrine causes giving rise to preterm labour include hyperthyroidism (Mestman *et al*, 1974), hyperparathyroidism (Johnstone, 1972) and hyperadrenocorticism (Grimes *et al*, 1973).

Trauma and injuries to the abdomen and gravid uterus would obviously induce preterm labour.

Infection, either clinical or subclinical, is considered to play a role in a significant proportion of cases of preterm labour and delivery. Lamont *et al* (1986) highlighted the association between abnormal colonization of the genital tract and preterm labour. Chorioamnitis has been estimated to account for up to 25% of preterm deliveries (Guziac and Winn, 1985).

Smoking is responsible for 20% of all low birth weight deliveries, 8% of all preterm births, and 5% of all perinatal deaths. Recent study suggested that 2 maternal metabolic genes, CYP1A1 and GSTT1, may modify the effect of cigarette smoking on birth weight. In this study, the mean reduction in birth weight for infants of smoking mothers was 377 g. (Wang *et al*, 2002).

Likewise, the use of narcotics and drugs during pregnancy also increases preterm births. Ney *et al* (1990) reported a positive urine toxicology screening in 17% of women with suspected preterm labour compared to 3% in uncomplicated term patients. Similar findings had previously been reported by MacGregor *et al* (1987).

Fetal abnormalities are associated with preterm labour and fetal death in utero usually results in delivery of the fetus within two to three weeks. (Lettieri *et al*, 1993). Antepartum hemorrhage, from placenta praevia or abruptio placenta, is associated with an increased incidence of preterm labour (Schneider, 1994).

Uterine factors such as overdistension of the uterus from polyhydramnios or multiple pregnancies often produce increased uterine activity and result in preterm labour (Gill *et al*, 1999).

In women with congenital malformation of the uterus, the incidence of preterm labour may be as high as 20%, if the pregnancy continues beyond 20 weeks' gestation. 16% of patients who had spontaneous preterm labour, without any obvious complication of pregnancy, were found to have congenital fundal abnormalities of the uterus and an additional 8% had incompetent cervix (Craig, 1974). These were ultrasonographically demonstrable in the puerperium compared to women delivering at term who had no such demonstrable anomalies.

Spontaneous rupture of the membranes precedes the onset of labour in a high percentage of preterm deliveries (Conon, 1994). In a study by Walker and Patel (1987) of pregnancies ending spontaneously between 20 and 28 weeks, spontaneous preterm rupture of membrane (PROM) was the principal precipitating obstetric factor in 14% of cases. The mechanism whereby the amniotic membranes rupture pretermly in some women is not entirely clear.

Mechanical dilatation of cervix greater than 8 mm has been held responsible for the increased risk of preterm births seen in women who had previously induced

abortions (Wyn *et al*, 1977). With the recent enthusiasm and recommendation for dilatation and evacuation for induced abortion up to 14 to 16 weeks' gestation, cervical trauma and incompetence may constitute a significant factor accounting for preterm labour in the future.

A definitely avoidable iatrogenic cause of preterm labour is elective delivery prior to term because of an error in estimated gestational age. Greater reliance on fetal ultrasonographic cephalometry and tests of fetal pulmonary maturity have greatly improved accuracy of estimating gestational age but have not totally eliminated the iatrogenic causes of preterm births.

Table 1 Etiological factors of preterm deliveries

Maternal	Fetoplacenta	Uterine	Iatrogenic
• Systemic illness • Endocrine causes • Trauma/injuries to abdomen • Infection	• Fetal abnormalities • Antepartum haemorrhage	• Polyhydromnios • Multiple pregnancy • Congenital malformation of uterus • Spontaneous rupture of membrane • Previous D&C	• Error in estimated gestational age

1.2.2.3 OTHER FACTORS ASSOCIATED WITH PRETERM DELIVERY

A number of epidemiologic factors have been associated with an increased frequency of preterm labour but such associations do not necessarily indicate a direct cause relationship.

Factors that have been associated with preterm labour, but where no cause and effect relationship can be clearly demonstrated, include race (Kaltreider *et al*, 1980), age, social environment (Chase *et al*, 1977), socio-economic status (Lumley, 1993), marital status (White *et al*, 1986), maternal stress, parity, previous abortion and preterm labour, previous fetal and neonatal deaths and previous bleeding and isoimmunization (Newton and Hunt, 1984). Low birth weights (both term and preterm) were most common among the first and after the fourth pregnancy (Prat *et al*, 1977).

Black women have a higher incidence of preterm birth (18.6%) than white women (8.3%) (Monthly Vital Statistic Report, United States Department of Health, Education and Welfare, 1973). In the Obstetrical Statistical Cooperative study from 1970 through 1976, non-whites had 7% of preterm low birth weight babies compared with 3.4% in whites (Kaltreider *et al*, 1980). Women of all racial

groups under 15 years of age had the highest incidence of preterm low birth weight infants while women aged 25-29 years had the lowest incidences. Boldman and Reeds (1977) noted that the incidence of low birth weight infants was increased in urban living, low per capita income, low per capita energy consumption, low newspaper circulation, lack of radio and television and low density of physicians. The "ward" (pavilion) patients had a 67-84% increase in incidence of preterm births compared with the "private" patients (Kaltreider and Kohl, 1980). Baird in 1977 found that the wives of labourers had the highest incidence of low birth weight babies, with the lowest incidence in wives of farmers. Wives of craftsmen and professionals were somewhere in between. Unmarried whites, but not non-whites, had a 90% higher incidence of preterm births than married whites (Kaltreider and Kohl al, 1980). However, illegitimate pregnancies had a higher incidence of low birth weight babies (term and preterm) for all racial groups (Pratt *et al*, 1977).

1.3 COMPLICATIONS OF PRETERM BABIES

Complications of preterm delivery of infants are due to immaturity of organs, blood system, immune system etc. Complications include patent ductus arteriosus, pulmonary air leaks, bronchopulmonary dysplasia, retinopathy of

prematurity, sepsis, anaemia and many others. However, the present study only focused on neonatal sepsis.

1.3.1 NEONATAL SEPSIS

Despite major advances in neonatology during the past few decades, many infants still develop life-threatening infections during the first month of life. The increasing populations of very low birth weight (VLBW) preterm infants who may now a days survive due to improved neonatal care, represent the group at highest risk for neonatal infection (Ishani, 2002).

Infection, either as a primary pathology or a complication of other illness, is a major cause of neonatal mortality and morbidity throughout the world. Neonatal bacteraemia is estimated to occur in 1-8 infants per 1000 live births and the incidence of neonatal bacterial meningitis is in the order of four infants per 10,000 live births (Klein *et al*, 1995). A comprehensive study of systemic bacterial and fungal infections in neonatal units in Australia showed incidence figures of 2.2 per 1000 live births for early-onset (<48 hours of birth) sepsis and 4.4 per 1000 live births for late-onset (>48 hours of birth) sepsis. The mortality rate for early-onset sepsis was 15% and for late-onset sepsis 9% (Issac, 1995). Among very low

birth weight babies undergoing prolonged intensive care, the rate of culture-proven sepsis may be as high as 30% with a mortality rate of 30% (Philip, 1994). Additionally, 20-30% of the survivors of neonatal bacteraemia and 40% or more of those with meningitis exhibit neurological sequelae (Klein *et al*, 1995).

In the developing world neonatal sepsis is a greater problem. A recent study from Malaysia reported rates of neonatal sepsis of 5-10% with case fatality rates of 23-52%. Septicaemia accounted for 11-30% of all neonatal deaths (Boo and Chor, 1994). In Hospital Kota Bharu, Nor Azmi and Noorizan (2002) reported that the nosocomial sepsis rate was 4.8 episode per 100 babies admitted and the mortality rate was 24%. Among neonates admitted to NICU, 30 (5.4%) in Hospital Kuala Terengganu (HKT) and 65 (3.6%) in Hospital Universiti Sains Malaysia (HUSM) had nosocomial infection. The number of neonates with predisposing factors for infection (HUSM 100%, HKT 73.3%) was higher in HUSM compared with HKT (Wan Hanifah and Quah, 2000).

Preterm delivery was the most important risk factor for both mortality and morbidity due to infections. In Trinidad, the most common cause of neonatal death was infection due to prematurity (43.9%) (Orret and Simon, 2001).

The risk of neonatal sepsis was 4-10 times higher in low birth weight infants than in full term neonates and varies inversely with gestational age (Wilson, 1990). In

USA, the prevalence of sepsis is 1 in 250 preterm newborns versus 1 in 15000 full term newborns (Lott and Kilb, 1992).

In Hospital Universiti Sains Malaysia, Halder et al (1999) did a study on 92 babies weighing 1000 – 1499 g admitted in NICU. The study showed an incidence of nosocomial sepsis in HUSM was 32.6% (30 out of 92) of whom 43.6% (13 out of 30) died.

Many studies have documented an increased rate of infection in preterm babies during the first few weeks of life. For example, the incidence of necrotizing enterocolitis (NEC), which may be an infectious process in at least some circumstances, is increased in preterm babies. A study done by Wu *et al* (2002) has found the incidence of perforated NEC in ELBW (extremely low birth weight) preterm babies was 5.1% and the overall survival was 37.5%.

Preterm babies are also endangered by generalized fungal infection due to their immature immune system. Laskus (1998) has done a study in 150 preterm babies of mothers with vaginal yeast colonization. It was found that Candida septicaemia developed one or two weeks after birth mainly in infant with birth weight below 1000 gram.

There is general agreement that preterm infants have more frequent and more severe lower respiratory tract infections than term infants especially those that requires mechanical ventilation (Pandya *et al*, 2001). This pattern is thought to result from immature local and systemic host defenses.

1.3.1.1 CLASSIFICATION OF NEONATAL SEPSIS

Classification of neonatal sepsis is useful as it explains common principles of causation, presentation and treatment. One of the most helpful classifications is given below.

1.3.1.1.1 EARLY ONSET SEPSIS (EOS)

Infections presenting within the first 48 hours of life are generally classified as early-onset infections although definitions range from 24 hours to 7 days. This category of infection is commonly caused by microorganisms, which are acquired from the mother before or during birth (vertically transmitted and perinatally

acquired). These organisms include group B streptococcus, *E. coli, Klebsiella, Listeria mònocytogenes* and nontypable *H.influenzae* (Ishani R, 2002).

1.3.1.1.2 LATE ONSET SEPSIS (LOS)

Infections presenting after 48 hours of age are considered late-onset infections and are generally caused by microorganisms acquired from the environment rather than from the mother (nosocomial and horizontally transmitted). In addition to bacteremia, hematogenous seeding may result in focal infections such as meningitis, osteomyelitis (group B *Streptococcus*, *Staphylococcus aureus*), arthritis (*gonococcus, S. aureus, Candida albicans*, gram-negative bacteria) and urinary tract infection (gram-negative bacteria) (Ishani R, 2002).

1.3.1.2 CLINICAL MANIFESTATIONS OF SEPSIS

The signs and symptoms of sepsis are usually subtle and non-specific. Infected foetuses may demonstrate pronounced tachycardia or decreased beat-to-beat

variability on foetal heart monitoring, be depressed at birth, require resuscitation and have low Apgar scores (Ishani R, 2002).

Lethargy, decreased alertness and responsiveness and decreased activity are common early features of neonatal sepsis. Both hypothermia and hyperthermia are recognized manifestations of infection. Hypoglycaemia or more commonly hyperglycaemia is frequently noted. Systemic hypotension is often associated with severe infection (Ishani R, 2002).

Respiratory symptoms ranging from apnoea, respiratory distress and need for assisted ventilation, occur in more than half of infants with culture proven sepsis. Other features include abdominal distension, feeding intolerance, increasing gastric aspirate, vomiting and hepatosplenomegaly. Seizures require prompt investigation for neonatal meningitis. Virtually all signs and symptoms attributable to neonatal infection could be indicative of conditions other than systemic infection (Ishani R, 2002).

1.3.1.3 DIAGNOSIS OF NEONATAL SEPSIS

Diagnosis of neonatal sepsis is based on the history, clinical features, and identification of the organisms. Laboratory evaluation assists in the diagnosis and

confirmation of infection. Positive cultures of blood, cerebrospinal fluid or urine are the gold standards for confirming infection. However, in a considerable proportion of neonates at risk of infection, culture results may be influenced by previous antibiotic exposure (Ishani R, 2002).

Blood is the most often examined body fluid for suspected sepsis. A positive blood culture may not always indicate true bacteraemia. Distinguishing true bacteraemia from contamination is a common problem encountered in VLBW preterm infants with suspected late onset sepsis. When an unusual organism or one with low virulence is isolated from blood, the clinician is in a dilemma about the significance of the culture result. The presence of the same pathogen in both bottles of a blood culture set and multiple positive blood cultures with the same organism, increase the likelihood of true bacteraemia (Ishani R, 2002).

Urinary tract infections (UTI) are relatively uncommon among infants with early onset sepsis but common among those with late onset sepsis. Urine should be cultured whenever there is suspicion of late onset sepsis. The best method of obtaining urine for culture is via suprapubic aspiration of the bladder. A bag specimen, if negative, is adequate to exclude UTI, but positive results should be confirmed by culture of a suprapubic sample (Ishani R, 2002).

The neonatal period carries the highest risk of meningitis of all age groups. Although meningitis represents a rare infection (4-10 per 10,000 live births), the high mortality rate and the substantial risk of adverse neurological sequelae warrant an aggressive approach to exclude this possibility. Some neonatologists limit lumbar puncture (LP) to infants who have a positive blood culture or who demonstrate symptoms specific to the central nervous system. However, up to 61% of infants with meningitis do not have concomitant bacteraemia. Lumbar puncture is a low-risk procedure and the consequences of missed or delayed diagnosis of meningitis are considerable (Wiswell T, 1995).

The value of surface swabs in the evaluation of neonatal sepsis is questionable. Cultures from different sites such as skin surface, gastric fluid, ear canal, umbilical cord and tracheal aspirates have been included in the septic screening. Surface swabs are informative about colonization, but not necessarily of infection. However, several studies have shown that the results of surface cultures are limited in the diagnosis of neonatal sepsis (Jolley, 1993).

1.4 NEONATAL IMMUNE SYSTEM

Increased susceptibility of preterm infant to infection is a result of many factors. The most important one is an immature immune system. In addition frequent

need for invasive procedures in the nursery complicates the problem. Optimal supportive care of the preterm infants involves frequent interruption of skin and mucosal surfaces with foreign bodies such as umbilical and other intravascular cathethers, chest tubes, heel punctures and endotracheal tubes. These procedures create port of entry for ordinarily noninvasive organisms such as *Staphylococcus aureus,* gram-negative enteric bacteria and *Candida albicans*. Once these organisms have gained access to the blood they may produce systemic infection, owing to the decreased ability of the preterm infant's host defenses to abort invasion (Ishani R, 2002).

Neonatal host defenses are immunologically immature and therefore contribute substantially to the high incidence of overwhelming sepsis (Mitchell, 1989). The immune system consists of two major components; innate and specific immune response (Roit *et al*, 2002).

1.4.1 THE INNATE IMMUNE SYSTEM OF NEONATES

1.4.1.1 THE PHAGOCYTE SYSTEM

Phagocytes develop early in gestation and can be seen in blood islands at the yolk sac stage of embryogenesis. Granulocytes and monocytes are found in the liver

and spleen at two and four months of gestation, respectively. By the fifth month, granulopoiesis takes place, primarily in the bone marrow (Moore et al, 1993). Polymorphonuclear leukocytes (PMN) are released from the bone marrow and circulate for about 6 hours before migrating into the tissues.

The function of neutrophils in host defense against infection includes adherence to capillary endothelium, diapedesis through capillary walls into the tissues, chemotaxis or directional migration to areas of inflammation, and phagocytosis and killing of microorganisms (Roit *et al*, 2002).

Absolute numbers of circulating neutrophils are increased in the neonate (both term and preterm) but the reserve or storage pool available in times of challenge is reduced and easily depleted and such depletion is associated with a higher mortality during sepsis (Erdman *et al*, 1982; Christen *et al*, 1986). In addition, neonatal neutrophils show a number of functional deficiencies, for example in chemotaxis, phagocytosis and bactericidal killing, particularly when put under stress such as hypoxia or sepsis (Hajjar, 1990; Carr *et al*, 1992).

In 1969, Dosset *et al* reported that fresh whole blood of the newborn had less bactericidal activity for E Coli than maternal whole blood. McCracken and Eichenwald (1971), using adult sera, found that term and preterm newborns PMN (polymorphonuclear leukocytes) reduced NBT (Nitrobluetetrazolium test) dye

normally. The studies reported by Forman and Stiehm (1969) with PMN from healthy term and low birth weight neonates showed similar results.

However, some other studies show conflicting findings. Park *et al* (1970) and Humbert *et al* (1969) reported increased NBT dye reduction in newborn PMN at rest and during phagocytosis.

Anderson *et al* (1979) studied NBT dye reduction and hexose monophosphate shunt activity in PMN from healthy term and preterm infants and infants with suspected or documented sepsis. Significantly lower unstimulated NBT dye reduction and lower stimulated hexose monophosphate shunt activity were found in PMN from newborns with sepsis. There was no correlation between NBT dye reduction or hexose monophosphate shunt activity and gestational age.

Upon activation, neutrophils release bactericidal/permability-increasing protein (BPI) from their azurophil granules. BPI selectively binds to lipopolysaccharide (LPS) on gram-negative bacteria and induces their death. Irmeli et al (2002) found that the ability of neutrophils to release BPI was lower in preterm babies compared to the term group (8.8 versus 15.9ng/10^6 neutrophils), which suggested that the neutrophil function was lower in preterm than in term babies.

1.4.1.2 THE COMPLEMENT SYSTEM

Complement is a collective term used to designate a group of plasma and cell membrane proteins that play a key role in the host defense process (Stites *et al*, 1997).

The complement system consists of approximately 30 serum molecules, which are nearly 10% of the total serum proteins. In evolutionary terms, this system is very ancient and antedates the development of the adaptive immune system. The functions of the system include control of inflammatory reactions and chemotaxis, clearance of immune complexes, cellular activation and antimicrobial defense. The system also plays a role in the development of antibody responses and is a major effector in immunopathological diseases (Roit *et al*, 2001)

1.4.1.2.1 COMPLEMENT SYNTHESIS IN THE FOETUS

The biosynthesis of C (complement) proteins begins early in human foetal development with the appearance of many C components before 18-20 weeks of gestation (Coltan and Goldberger, 1979). The foetal liver synthesizes C4 and C2

as early as 8 weeks of gestation and C3 by 14 weeks of gestation. By the end of the first trimester, C1 and C5 are being produced (Kohler, 1973). Adinolfi and Gardner (1977) found C3 and C4 in the foetuses greater than 14 weeks gestation, but Gitlin and Biasucci (1969) detected C3 in the serum as early as 5 $^{1}/_{2}$ weeks.

Serum complement levels in the foetus remain low until early in the third trimester, at which they start to increase significantly. The level of C3, for example, rises rapidly at 26 to 28 weeks of gestation to approximately 50 – 70% of adult levels (Adinolphi, 1977). The mechanism for this sudden change in rate of foetal synthesis is not well understood.

1.4.1.2.2 COMPLEMENT LEVELS IN THE NEWBORN

Sawyer and colleagues (1971) compared the levels of classical C pathway total hemolytic activity (CH50), C1q, C2 and C3 in the sera of normal full term infants with those of low birth weight infants. The values of all classical pathway parameters were much lower in preterm than in full term infants. Another study by Wolach et al (1997) showed that preterm infants had lower complement activity and complement component levels than full term infants. In this study,

complement levels correlated significantly with gestational age but not with birth weight, type of delivery or gender.

1.4.2 SPECIFIC IMMUNE RESPONSE IN NEONATES

1.4.2.1 HUMORAL IMMUNITY

Immunoglobulins are the critical ingredients at every stage of a humoral immune response. The immunoglobulins that are secreted function as antibodies, traveling through the tissue fluids to seek out and bind to the specific antigens that triggered their production. Five distinct classes of immunoglobulin molecules are recognized in most higher mammals, namely IgG, IgM, IgD, IgA and IgE (Roit *et al*, 2001).

Van Furth *et al.* (1965) studied immunoglobulin synthesis at various stages of gestation, using various experimental techniques. The study showed that the human foetus is capable of synthesizing IgG and IgM at about 20th week of gestation and the main site of immunoglobulin synthesis in this phase is spleen. A low level of serum IgM synthesis to about one tenth of adult level was demonstrated. Furthermore, cells containing IgG and IgM were demonstrated by

immunofluorescence in foetal peripheral blood at 21st week of gestation. IgA synthesis was not demonstrated until after the 30th week of gestation and IgD synthesis was not found at all. These results were later confirmed by Lawton *et al* (1972).

A few more studies also demonstrated that a foetus is capable of synthesizing IgM and IgG from 20th week of gestation in response to congenital infection (Silverstein and Lukes, 1962; Alfres *et al*, 1974). A distinct from Van Furth et al, Gitlin and Biasucci (1969) demonstrated that synthesis of IgM could start half way through 11th week of gestation.

Synthesis of IgA in the foetal period remains unclear. Van Furth *et al* (1965) and Howard and Lawton (1977) failed to demonstrate IgA synthesis in any foetal tissues they examined. However, Gitlin and Biasucci (1969) found a low level of serum IgA in 6.5 weeks embryos.

1.4.2.1.1 TRANSPLACENTAL TRANSMISSION OF IMMUNOGLOBULINS

It is suggested that only IgG molecules cross the human placenta (Roit *et al*, 2001). Transfer of IgG via the placenta begins in the 20th week of gestation, but

most of the transfer occurs after 31st week of gestation. Immunologic protection due to maternal IgG is directly correlated with gestational age, and infants born before 31st week are at a disadvantage because of their lower levels of maternal IgG (Wendy, 2001).

1.4.2.1.2 LEVELS OF IMMUNOGLOBULIN

Conway *et al* (1985) did a study on 64 preterm infants and they found that IgG values at birth ranged from 2.6 g/l to 14.4 g/l. Multiple regression analysis showed that the concentration of IgG at birth was affected by gestational age and birth weight. IgM level ranged from 0 – 0.6 g/l and IgA was found in only 10 of 57 samples. There was no detectable effect of gestational age or birth weight on the concentration of IgM and IgA at birth.

A study by Ballow *et al* (1986), on very low birth weight (<1.5 kg) preterm infants (VLBW) also showed that the concentration of IgG at birth was affected by gestational age or birth weight or both, but no detectable effect on the concentration of IgA and IgM. When comparing the levels of immunoglobulin in preterm and term babies, Marisa and Arthur (1986) concluded that serum IgG was higher in term than preterm babies whereas there were no significance difference between the two groups for serum IgM and IgA. They also follow-up

the patients for 1 year and found that the preterm group showed a mean occurrence of sepsis of 4.2 episodes per year per child, and the full-term group at 3.7 episodes per year per child.

1.4.2.2 CELL MEDIATED IMMUNITY

Thymus develops embryologically as an outgrowth from the third and fourth pharyngeal pouches between the sixth and seventh weeks of gestation. Lymphocytes destined to become T cells appear among epithelial cells during the weeks 9 and 10. However, it is not until the tenth week of gestation that the cortex and medulla of the thymus begin to demarcate. E-rosetting and mitogen responsive cells appear in increasing numbers in the peripheral blood and spleen after 12 weeks of gestation and reach nearly adult levels by 20 weeks' of gestation (Avroy and Richard, 1992).

It is well known that the total lymphocyte count is increased in newborn infant compared with adults (Wara and Barret, 1979). The percentage of T lymphocytes in the newborn has been variously reported as normal or decreased in term infants, although the absolute values are comparable with, or higher than adult values (Sandahl *et al*, 1976). One study has compared preterm with term infants,

but showed no difference in the number of T or CD19 (B cell)s (Campbell *et al*, 1974). Another study also compared lymphocyte subsets in the preterm and term infant. The study also showed that there was no significant difference in absolute numbers of lymphocyte subsets between those two groups (Thomas and Linch, 1983).

Lymphocyte subsets in the cord blood of full term babies were compared with that of adult blood and were found to be significantly different in numbers and percentages of lymphocyte subpopulations. The mean absolute number of cord T cells, including both CD4 and CD8 T cells subsets were significantly greater than adult levels and the mean percentage and absolute numbers of CD16/56 (NK cell)s were also higher in cord blood (Beck and Lam-Po-Tang, 1994).

2. OBJECTIVES

General objective

1. To identify immunological parameters of preterm babies from the cord blood – pilot study.

Specific objectives

1. To compare the immunological parameters (immunoglobulin level, complement level, lymphocyte subset and neutrophil function) in the cord blood between preterm and term babies– pilot study.

2. To correlate immunological parameters in cord blood with occurrence of sepsis within 2 weeks post delivery in preterm babies. – pilot study.

3. To evaluate the effects of antenatal steroids on the immunological parameters in preterm babies - pilot study.

3. METHODOLOGY

3.1 STUDY DESIGN

This is a cross sectional pilot study, which was conducted in Labour Room and Immunology Laboratory HUSM, from April 2003 till June 2004 using convenient sampling method. After the proposal has been presented and accepted by the ethical committee, this study was done, and completed as planned.

3.2 PATIENTS

3.2.1 INCLUSION CRITERIA

1. Preterm babies born via spontaneous vaginal delivery and lower segment caesarian section (LSCS) in HUSM. A preterm baby is

defined as live born delivered between 22 to 37 weeks from the first day of last menstrual period (Kliegman, 1996).

2. Term babies born in HUSM via spontaneous vaginal delivery and lower segment caesarian section. Term baby is defined as baby born from 37 to 42 weeks of pregnancy (Maternal, Newborn & Early Childhood Development Resource Center, 2002)

The gestational age of an infant is determined by assessment from the mother's menstrual history or ultrasound.

Sepsis is defined by clinically diagnosed infection and/or positive laboratory investigations.

3.2.2 EXCLUSION CRITERIA

a. Preterm and term babies with presumed infection. Presumed infection is defined as presence of clinical sign and symptom of infection (for example lethargy, unresponsiveness, pallor and tachycardia) without confirmation by laboratory investigation.

b. Preterm and term babies with any congenital abnormalities.

Congenital abnormalities include 5 categories (Jones, 1996):

1 Syndrome e.g.: Down syndrome, Edward syndrome

2 Malformation e.g.: Congenital Heart Disease, Diaphragmatic Hernia

3 Deformation e.g.: Club foot

4 Disruption e.g.: Amniotic band

5 Association e.g.: VATER, VACTERL

c. Preterm and term babies from mother with any medical illness.

Medical illness includes:

1 Hypertensive disorder

2 Endocrine disorders e.g.: Diabetes mellitus, thyrotoxicosis

3.2.3 CONSENT

All the candidates were consulted about the study process and the cord blood was collected after written consent.

3.3 STUDY METHOD

Blood samples

Five ml of blood was taken from the cord blood of both preterm and term babies for the following investigations:

1. Two ml in plain container: for detection of serum immunoglobulins and complement levels (C3 and C4).
2. One ml in heparinised container: for neutrophil function test.
3. Two ml in EDTA container: for determination of lymphocyte subsets.

The details about the clinical features of infection, the causative organisms and mothers' history during pregnancy were collected from the medical records. Type of feeding was also included in the data.

3.4 METHOD OF THE TEST

3.4.1 DETECTION OF IMMUNOGLOBULIN BY IMMUNOTURBIDOMETRY

3.4.1.1. Reagents

The kit (Turbox) (Orion Diagnostica, Espoo, Finland) contains

IgG Buffer

IgG Blank Buffer

IgG antiserum reagent

Calibrator

Magnetic card.

Internal quality control

3.4.1.2 Apparatus

Turbox analyzer

Cuvette glass

3.4.1.3 Procedures

The principle of the test is antiserum to IgG is diluted in a buffer and added to an aliquot of patient serum. The light scattering caused by antigen-antibody complex is measured after incubation. The resulting light scattering is directly proportional to the IgG concentration in the sample.

3.4.1.4 Preparation of the reagents:

Antiserum-buffer solution: pipette 500µl antiserum reagent into the reagent buffer .

The blank buffer is ready to use

Calibrator is diluted 1:51 with 0.9% NaCl.

3.4.1.5 Sample preparation:

Fresh serum or plasma was used in this method. The samples were diluted 1:51 with 0.9% NaCl. Turbid sample was centrifuged before the assay was performed.

A separate sample blank for each sample as well as calibrator blank for calibrator was prepared. The calibrator was prepared in duplicate. Then all these samples and calibrators were pipetted into different cuvettes (µl):

	Calibrator Blank	Calibrator test	Sample blank	Sample test
Sample (µl)	-	-	20	20
Calibrator (µl)	20	20	-	-
Blank buffer (µl)	500	-	500	-
Antiserum dilution (µl)	-	500	-	500

All these sample, calibrator, buffer and antiserum in the cuvette were mixed by shaking gently and allowed to stand for 30 minutes at room temperature (18-25 C). Each cuvette was mixed again gently before measuring with Turbox analyzer.

The same procedures for measurements of IgA and IgM was done but by using 150 µl and 50µl of sample and calibrator respectively.

3.4.2 NEUTROPHIL FUNCTION TEST BY NITROBLUE TETRAZOLIUM TEST (NBT)

3.4.2.1. Reagents

NBT reduction kit (Sigma Diagnostics, St. Louis, USA) contains

NBT vial (Nitroblue tetrazolium, 1 mg, lyophilized)

Siliconized glass vials with heparin, 20 units for collecting 1 ml whole blood

Vials, glass with caps

Accustain wright stain

Stimulant (bacterial extracts, lyophilized)

3.4.2.2 Apparatus

Waterbath

Glass slide

Plastic pipette

3.4.2.3. Procedure

NBT solution was prepared by reconstituting NBT vial with 1.0 ml distilled water. Stimulant solution was prepared by reconstituting Stimulant with 1.5 ml distilled water.

Unstimulated procedure :-

This unstimulated test was done in order to detect metabolic defects of neutrophil function.

With a plastic pipette, 0.12 ml NBT Solution was transferred to a vial. Then 0.2 ml well mixed heparinized blood was added. The vial was mixed gently by tilting and rolling slightly. Then it was cap tightly and was incubate at 37°C for 5 minutes. After that the vial was removed from incubation and allowed to stand at room temperature for 10 minutes. Heparinized blood-NBT mixture was mixed again by rolling gently. With a plastic pipette, 50-75µl of mixture was transferred onto clean glass slide. Smear was prepared moderately

thick in order to reduce mechanical damage to formazan-containing cells, which were fragile. Smear was permitted to air dry.

Then the smear was treated with Accustain Wright Stain as follows: dry smear was flooded with 1 ml of stain for 15 seconds. One ml of distilled water was added to the flooded smear and allowed to stand for 30 seconds. Smear was rinsed with water, allowed to drain and blotted or air dried.

Stained smear was scanned using oil emersion objective and a total of 100 or more neutrophils were counted. Those neutrophils showed formazan deposits were recorded as positive. The neutrophil must be whole with intact cell membrane, must be solitary and must contain formazan deposits as large irregularly shaped, discrete masses.

Stimulated procedure:-

This procedure was performed as a positive control in conjunction with unstimulated procedure.

0.1 ml NBT Solution was transferred into a vial. With a plastic pipette 0.05 ml heparinized blood and 5 µl Stimulant Solution were added. It was then mixed gently by rolling the vial. The vial was cap tightly. Then the same procedures proceed as the unstimulated procedure.

The normal range for the unstimulated procedure: 2-17% with the mean of 9% and for the stimulated procedure 3 x of the unstimulated value.

3.4.3 DETERMINATION OF LYMPHOCYTE SUBSETS BY IMMUNOPHENOTYPING

3.4.3.1 Reagents

TriTEST CD3 FITC/CD19/CD45 PerCP kit

FACS lysing solution

3.4.3.2 Apparatus

Flowcytometer

Vortex mixer

Plastic pipette

3.4.3.3 Procedures

The principle of the test is when whole blood is added to the reagent the fluorochrome-labeled antibodies in the reagent bind specifically to leucocyte surface antigens. During acquisition, the cells travel past the laser beam and scatter the laser light. The stained cells fluoresce. These scatter and fluorescence signals, detected by the instrument, provide information about the cell's size, internal complexity and relative fluorescence intensity. TriTEST reagents employ fluorescence triggering, allowing direct fluorescence gating of the lymphocyte population to reduce contamination of unlysed or nucleated red blood cells in the gate.

Twenty µl of TriTEST CD3 FITC/CD19/CD45 PerCP was added in each of four test tubes. Then 50 µl whole blood was added in each

tube. The tube was cap and vortex gently to mix. These tubes were then incubated for 15 minutes in the dark place. After that 450µl Facs lysing solution was added in each tube. The tube was cap and vortex gently to mix. The tubes were then incubated again for another 15 minutes in the dark room at room temperature. After incubation, the tubes were read by flowcytometer.

3.4.4 DETERMINATION OF COMPLEMENT LEVELS (C3 AND C4) BY IMMUNOTURBIDOMETRY

3.4.4.1 Reagents

The kit (Turbox) (Orion Diagnostica, Espoo, Finland) contains

C3 Buffer

C3 Blank Buffer

C3 Antiserum Reagent (rabbit)

Calibrator.

Magnetic card

3.4.4.2 Apparatus

Turbox analyzer

Cuvette glass

3.4.4.3 Procedure

The principle of the test is anti-serum to C3 is diluted in buffer and added to an aliquot of patient serum. The light scattering caused by antigen-antibody-complexes is measured which is directly proportional to the C3 concentration in the sample.

A separate sample blank for each sample and a calibrator blank for each calibrator was prepared. The calibrator was prepared in duplicate. Then the mixture was pipetted into the cuvettes (µl):

	Calibrator Blank	Calibrator test	Sample blank	Sample test
Sample (µl)	-	-	25	25
Calibrator (µl)	25	25	-	-
Blank buffer (µl)	500	-	500	-
Anti-serum dilution (µl)	-	500	-	500

Then the cuvettes were mixed by shaking gently and allowed to stand for 30 minutes at room temperature (18-25° C). The mixture was measured by using the Turbox analyzer.

The same procedure was done for C4 by using 100 µl sample blank, sample test, calibrator blank and calibrator test.

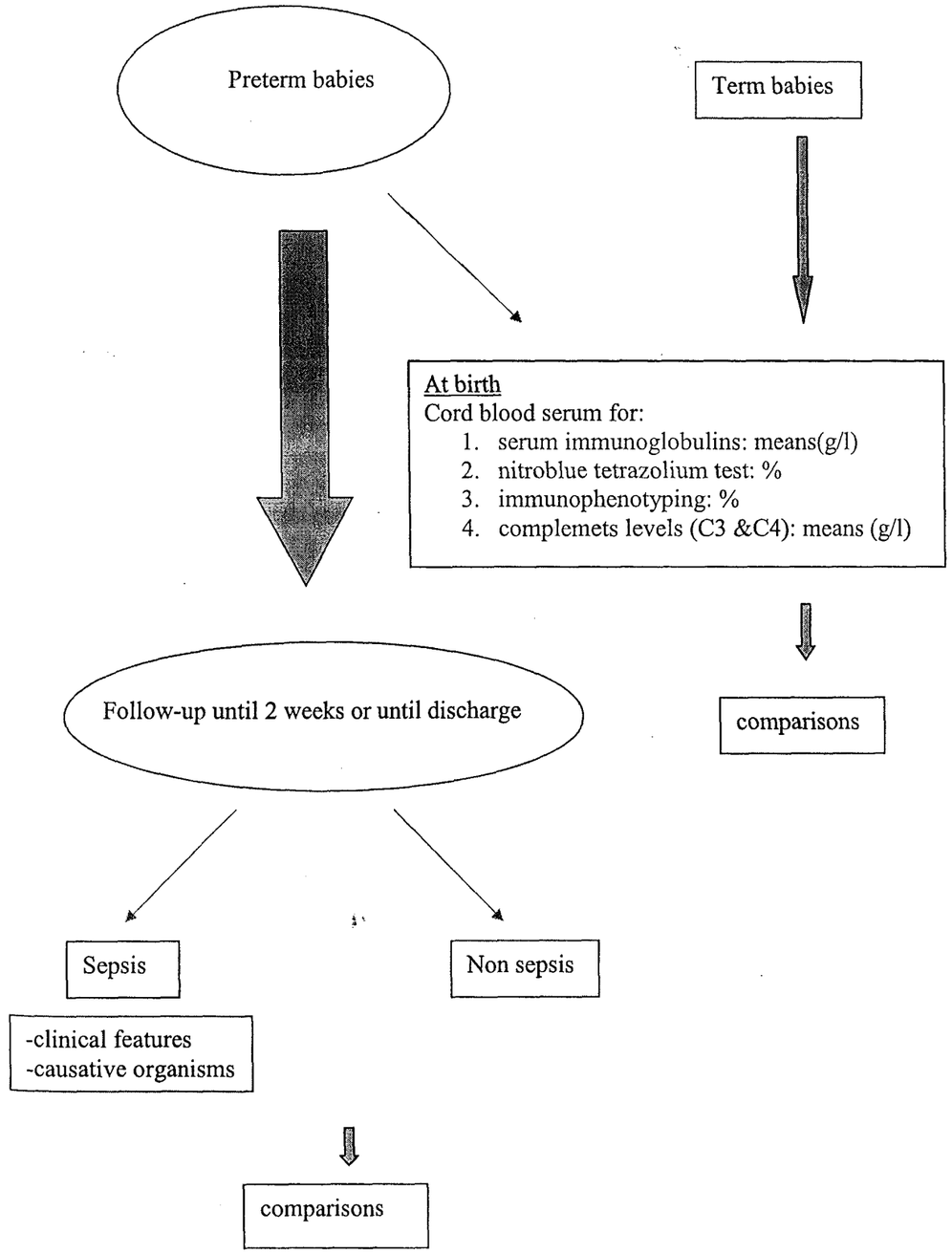
The blood investigations were done at birth by using cord blood. Then, we follow-up the babies while in the ward for 2 weeks of hospitalization or until discharged.

3.5 THE STATISTICAL ANALYSIS

All data were entered and analyzed by means of statistical software SPSS version 11.

Categorical variables were analyzed by using univariate analysis of Chi-square and Fisher' exact test and numerical variables by using independent t-test. Independent t-test analysis was also used for hypothesis test. Mann-Whitney test was used for not normally distributed variables. For association, single linear regression was used for numerical variables whereas multiple logistic regression was used for categorical variables. A p value of < 0.05 was considered as significant.

4.6 Flow chart of the study



4 RESULTS

4.1 SEX, TYPE OF FEEDING AND MODE OF DELIVERY

Cord blood samples from 36 preterm babies and an equal number of cord blood samples from term babies were taken and analyzed. The sex distribution of the study group and the control group is shown in table 4.1. There were more males than female in each group but the sex distribution was very similar in both groups (p value = 0.812).

There were more babies given formula milk in the preterm group than in the term group (63.9% versus 2.8%). This difference in feeding was statistically significance with a p value of < 0.001. These data are shown in table 4.1 and figure 4.1.

The mode of delivery is also shown in table 4.1. Seventy two point two percent of preterm babies were born via spontaneous vaginal delivery (SVD) and 27.8%

via lower segment caesarian section (LSCS) whereas in the group of term babies 100% were born via spontaneous vaginal deliveries (SVD). This difference in mode of delivery was statistically significant with a p value of 0.001.

Table 4.1 Frequency and percentage of sex, type of feeding and mode of delivery (MOD) in preterm and term babies.

	Preterm		Term		P value
	Frequency	%	Frequency	%	
1 Sex					
Male	20	55.6%	21	58.3%	0.812*
Female	16	44.4%	15	41.7%	
2 Feeding					
Formula milk	13	63.9%	1	2.8%	< 0.001*
Breast milk	23	36.1%	35	97.2%	
3 MOD					
SVD	26	72.2%	36	100%	0.001[0]
LSCS	10	27.8%	0	0%	

* Chi square

[0] Fisher exact

Significant at 95% CI when $p < 0.05$

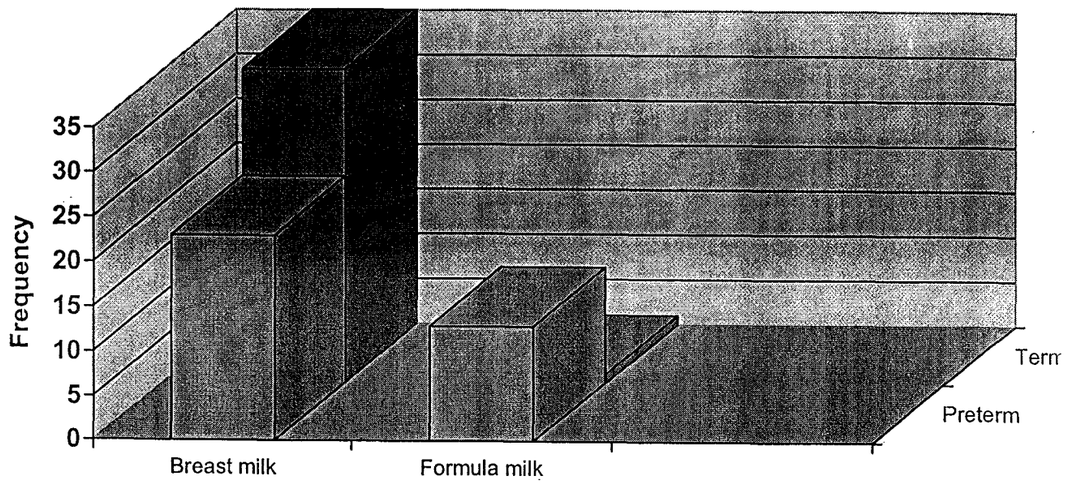


Figure 4.1 : Type of feeding in preterm and term babies.

4.2 GRAVIDITY AND PERIOD OF AMENORRHOEA OF MOTHERS AND WEIGHT OF BABIES

As shown in table 4.2, the gravidity of mothers of preterm babies ranged from gravida 1 to gravida 9 and that of mothers of term babies range from gravida 1 to gravida 10. The mean gravidity in the preterm babies was lower than that in the term group (3.17 versus 3.53). The difference in gravidity was not significant (p = 0.518).

The mean period of amenorrhoea (POA) of mothers of preterm babies was 34.47 weeks of gestation (range from 29 weeks to 36 weeks) and the mean POA of mothers of term babies was 38.78 weeks of gestation (range from 37 weeks to 41 weeks). This difference in period of amenorrhoea was statistically significant, with a p value of < 0.001. These data are shown in table 4.2 and figure 4.2.

The mean weight of preterm babies was 2.39 kg. The smallest baby in this group was 1.8 kg and the biggest was 2.99 kg. In term babies the mean weight was 3.18 kg. The weight of these term babies ranged between 2.41 kg to 4.06 kg. The p value was < 0.001. There was a significant difference of weight between the preterm and term babies (table 4.2).

Table 4.2 Mean (SD) of gravidity and period of amenorrhoea (POA) of mothers of preterm and term babies and weight of preterm and term babies.

	Preterm		Term		
	Mean	SD	Mean	SD	p value*
Gravidity	3.17	2.4	3.53	2.27	0.518
POA (weeks)	34.47	1.73	38.78	1.29	< 0.001
Weight (kg)	2.39	1.8	3.18	2.74	<0.001

* Independent T test

Significant at 95% CI when p value < 0.05

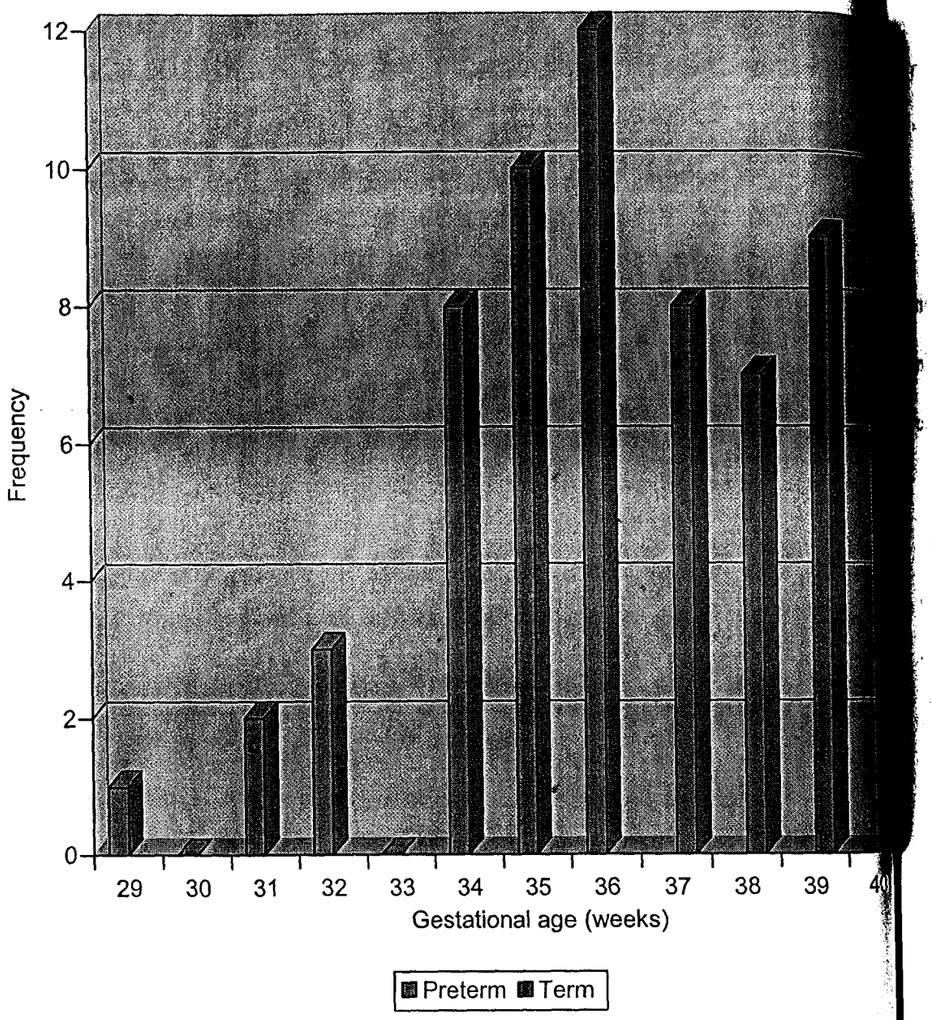


Figure 4.2 : Gestational age of preterm and term babies.

4.3 COMPARISON OF IMMUNOLOGICAL PARAMETERS IN THE CORD BLOOD OF PRETERM AND TERM BABIES

The comparison of immunological parameters between preterm and term babies is shown in tables 4.3 and 4.4. Table 4.3 shows the immunological parameters that were normally distributed and table 4.4 shows the not normally distributed immunological parameters (non parametric variables).

Among the most remarkable differences between the two groups, were those found for the complement factors, C3 and C4. Serum C3 level was significantly lower in preterm than in term babies with mean levels of 0.5114 versus 0.7192g/l. The p value for this difference was <0.001. Similarly C4 level was also significantly lower in preterm than in term babies (means of 0.07 versus 0.14g/l with $p < 0.001$).

As for the immunoglobulins, each IgG, IgA and IgM have lower mean or median serum levels in the preterm group than in the term group. The mean serum IgG level for preterm and term babies was 9.5583g/l and 14.2806g/l respectively. The level of IgA was detectable in both preterm and term babies. The mean IgA level for preterm babies was 0.210g/l and for term babies the mean was 0.225g/l. For serum IgM level, which was a non parametric variable, the median for preterm and term babies was 0.1g/l and 0.2g/l respectively. The differences between both

groups were most marked for IgG and IgM with p values of less than 0.001 for each. For IgA, there was a less marked difference between the two groups than for IgG and IgM. The difference however still reached significance (p value = 0.035).

For neutrophil function, there was a significant difference of NBT reduction between preterm and term babies. This was supported by a p value of 0.001. In preterm babies, the median for NBT reduction was 7.5% while for term babies the median was 12.0%.

For lymphocyte subsets, CD3%, CD4% and CD16/56 (NK cell)% were each found to have lower mean or median percentages in the preterm group than in the term group. The mean CD 3 % for preterm and term babies was 65.2917% and 70.3153% respectively. For lymphocyte subset, only CD3% was significantly different in both groups. This was supported by a p value of 0.035.

The mean CD 4% for preterm babies was 43.0731% and for term babies, the mean was 46.4397% with a p value of 0.128. Similarly, CD16/56 (NK cell)%, which was a non parametric variable, was lower in preterm babies than in term babies (medians of 9.0% versus 10.0% with a p value of 0.566).

CD8% and CD19 (B cell)% were found to have a higher mean or median in the preterm group than in the term group. The mean CD19 (B cell)% for preterm and term babies was 20.1556% and 17.2581% respectively (p value = 0.065). The median of CD 8% was slightly higher in preterm babies than in term babies (22.7% versus 22.3% with p value = 0.928).

In summary, the NBT reduction, C3 level, C4 level, IgG level, IgA level, IgM level, CD3%, CD 4% and CD16/56 (NK) % cell were lower in preterm than in term babies. However only NBT reduction, C3 level, C4 level, IgG level, IgA level, IgM level and CD3% were significantly different between preterm and term babies whereas CD 4%, CD8%, CD19 (B cell)% and CD16/56 (NK)% cell percentages were not significantly different between the 2 groups.

Table 4.3 Immunological parameters in preterm and term babies

	Preterm Mean(SD) (n=36)	Term Mean (SD) (n=36)	Mean diff (95% CI)	T(df)	p value*
C3 (g/l)	0.5114 (0.0879)	0.7192 (0.0735)	0.2078 (0.1697 – 0.2459)	10.880 (70)	<0.001
C4 (g/l)	0.0769 (0.0229)	0.1369 (0.0287)	0.0600 (0.0478 - 0.0722)	9.790 (70)	<0.001
IgG (g/l)	9.5583 (2.3701)	14.2806 (2.1738)	4.7222 (3.6532 - 5.7913)	8.810 (70)	<0.001
IgA (g/l)	0.2100 (.0207)	0.2250 (0.0364)	0.0150 (0.0011 - 0.0289)	2.151 (70)	0.036
CD3%	65.2917 (10.8819)	70.3153 (8.8454)	5.0236 (0.3621 - 9.6851)	2.149 (70)	0.035
CD4%	43.0731 (10.4296)	46.4397 (7.9411)	3.3667 (-0.9907 - 7.7240)	1.541 (70)	0.128
CD19 (B cell) %	20.1556 (5.6535)	17.2581 (7.3508)	-2.8975 (-5.9800 - 0.1850)	-1.875 (70)	0.065

Independent T test, $p < 0.05$ significant at 95% CI

Table 4.4 Immunological parameters of non parametric variables in preterm and term babies

Variables	Preterm Median (IQR) (n=36)	Term Median (IQR) (n=36)	Z stat [a]	p value
IgM (g/l)	0.1 (0.02)	0.2 (0.04)	-5.585	<0.001
NBT (%)	7.5 (5.00)	12.0 (8.50)	-3.445	0.001
CD8 %	22.7 (10.89)	22.3 (6.48)	-0.090	0.928
CD16/56 (NK cell) %	9.0 (15.12)	10.0 (10.20)	-0.0574	0.566

a : Mann -Whitney Test

$p < 0.05$ significant at 95% CI

4.4 ASSOCIATION OF IMMUNOLOGICAL PARAMETERS IN THE CORD BLOOD WITH OCCURRENCE OF SEPSIS WITHIN 2 WEEKS POST DELIVERY IN PRETERM BABIES

4.4.1 FREQUENCY OF SEPSIS

As shown in figure 4.3, follow-up of the babies for 2 weeks after birth showed that sepsis occurred in 11.1% of preterm babies (4 out of 36). In all of them, the infection was thought to affect predominantly the respiratory system. All of the preterm babies who had been diagnosed as sepsis were culture negative sepsis but they were diagnosed clinically based on signs and symptoms of sepsis. There was no case of urinary infection, meningitis or serious systemic infection. These babies were treated with IV (intravenous) antibiotics and showed a good response to this therapy. No other explanation could be found for their signs and symptoms.

None of the term babies had infection within 2 weeks of follow-up. One out of 36 term babies was admitted in NICU due to shoulder dystocia. This baby was a large for gestational age baby with a birth weight of 4.06 kg. However, the

MOGTT (modified oral glucose intolerance test) of the mother was negative during antenatal follow-up. The babies had no signs or symptoms of sepsis

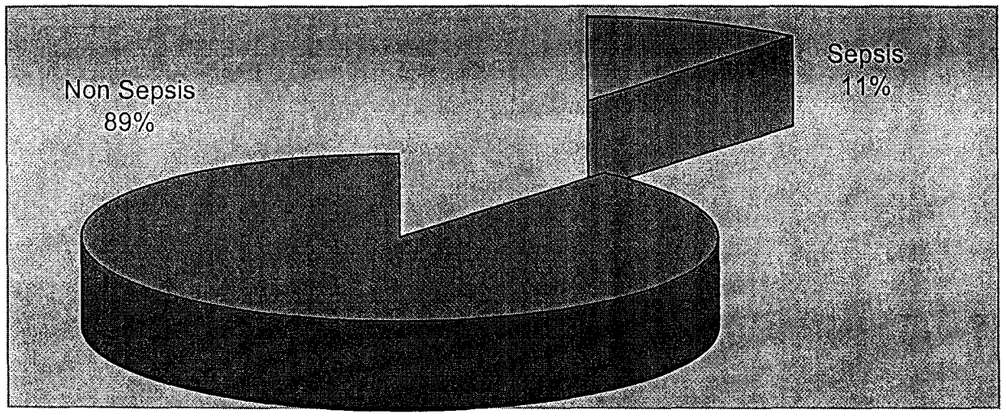


Figure 4.3 : Distribution of sepsis in preterm babies.

4.4.2 ASSOCIATION BETWEEN IMMUNOLOGICAL PARAMETERS AND SEPSIS IN PRETERM BABIES

The association between immunological parameters and sepsis in preterm babies was analyzed using the Mann Whitney Test. The result of this univariate analysis are shown in table 4.5. The NBT reduction and IgA level were found to be significantly different between the group of preterm babies with sepsis and that of preterm babies without sepsis.

For NBT reduction, the median for preterm babies with sepsis was significantly lower than for those without sepsis (3.50% versus 8.00% with p value = 0.017).

As for immunoglobulin levels, the median level of IgA level was significantly lower in preterm babies with sepsis than in preterm babies without sepsis (0.190 versus 0.210 g/l, p value = 0.007) whereas IgG and IgM levels were not different significantly between the 2 groups.

The complement factors (C3 and C4 levels) and lymphocyte subsets (CD3%, CD4%, CD8%, CD19 (B cell) % and CD16/56 (NK cell) were not significantly different between the 2 groups.

A multiple logistic regression test was performed but the results were very difficult to interpret. This was most likely due to small sample size of this pilot study.

In summary, using univariate analysis, NBT reduction and IgA level were significantly lower in preterm babies with sepsis than those without sepsis whereas C3 level, C4 level, IgG level, IgM level, CD3%, CD4%, CD8%, CD19 (B cell)% and CD16/56 (NK cell)% were not significantly different between the two groups.

Table 4.5 Association of sepsis with immunological parameters in preterm babies

Variables	Sepsis Median (IQR) (n=4)	Not sepsis Median (IQR) (n=32)	Z stat [a]	p value
NBT (%)	3.50 (1.75)	8.00 (4.00)	-2.33	0.017
C3 (g/l)	0.44 (0.24)	0.51 (0.13)	-0.93	0.366
C4 (g/l)	0.05 (0.04)	0.08 (0.03)	-1.70	0.092
IgG (g/l)	7.25 (6.20)	9.89 (3.35)	-1.13	0.269
IgA (g/l)	0.19 (0.02)	0.21(0.02)	-2.64	0.007
IgM (g/l)	0.14 (0.11)	0.11 (0.02)	-0.41	0.716
CD3 %	60.36 (21.88)	66.30 (17.30)	-0.86	0.421
CD4 %	41.98 (17.16)	43.30 (14.78)	-0.10	0.942
CD8 %	16.23 (7.62)	23.54 (11.95)	-2.01	0.051
CD19 (B cell)%	22.22(4.69)	20.14 (7.97)	-1.01	0.340
CD16/56 (NK cell)%	13.88 (23.34)	9.05 (11.10)	-0.02	0.981

a = Mann -Whitney Test

$p < 0.05$ significant at 95% CI

4.5 EFFECTS OF ANTENATAL DEXAMETHASONE ON IMMUNOLOGICAL PARAMETERS IN PRETERM BABIES

4.5.1 FREQUENCY OF IM DEXAMETHASONE GIVEN TO MOTHERS OF PRETERM BABIES

Dexamethasone was given to mothers of preterm babies for lung maturation. In this study, 24 out of 36 (66.7%) mothers of preterm babies were given dexamethasone (figure 4.4). 12 others (33.3%) were not given dexamethasone or given 2 doses of dexamethasone in less than 4 hours.

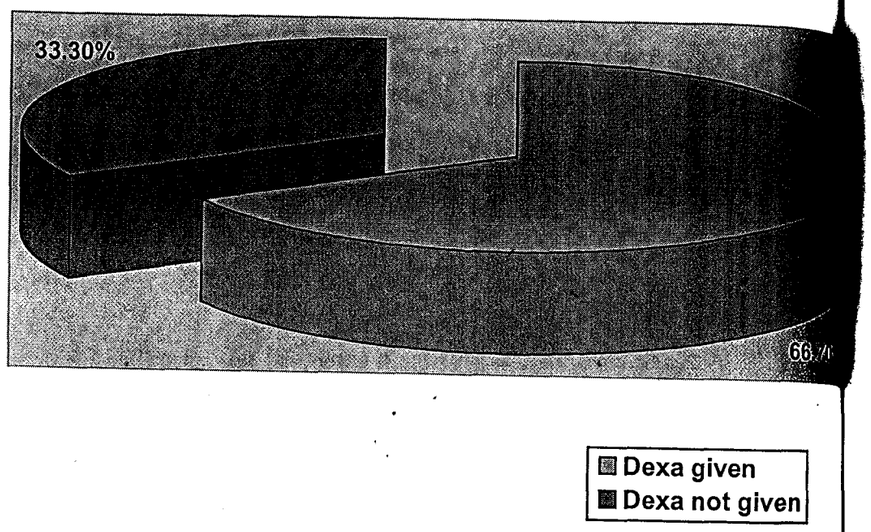


Figure 4.4 Distribution of dexamethasone given to mothers of preterm babies

4.5.2 EFFECT OF ANTENATAL DEXAMETHASONE ON IMMUNOLOGICAL PARAMETERS IN PRETERM BABIES.

The association between antenatal dexamethasone administration with immunological parameters was analyzed by using independent t test (table 4.6). However, none of these immunological parameters, were found to be statistically significantly different among babies born to mothers receiving dexamethasone and those born to mothers not receiving dexamethasone. There were trends towards a lower NBT reduction, a higher CD8% and a lower CD4% in the babies of mothers receiving dexamethasone.

Table 4.6 Association of antenatal dexamethasone with immunological parameters in preterm babies

	Dexa Mean (SD) (n = 24)	No dexa Mean (SD) (n = 12)	Mean diff (95% CI)	T(df)	p value*
NBT (%)	6.67 (2.959)	8.42 (4.08)	1.75	1.472 (34)	0.150
C3 (g/l)	0.499 (0.078)	0.537 (0.104)	0.038	1.229 (34)	0.227
C4 (g/l)	0.077 (0.024)	0.077 (0.245)	0.001	0.101 (34)	0.920
IgG (g/l)	9.358 (2.225)	9.958 (2.693)	0.600	0.711 (34)	0.482
IgA (g/l)	0.208 (0.198)	0.214 (0.023)	0.006	0.851 (34)	0.401
IgM (g/l)	0.109 (0.015)	0.118 (0.036)	0.010	1.117 (34)	0.398
CD3 %	65.758 (11.609)	64.358 (9.672)	-1.400	-0.359 (34)	0.722
CD4 %	41.688 (11.847)	45.843 (6.331)	4.155	1.131 (34)	0.179
CD8 %	24.500 (7.859)	20.756 (7.813)	-3.744	-1.350 (34)	0.186
CD19 (B cell)%	20.169 (4.965)	20.128 (7.082)	-0.041	-0.20 (34)	0.984
CD16/56 (NK cell)%	12.670 (9.013)	11.942 (7.791)	10.738	-0.239 (34)	0.813

* Independent T Test, $p < 0.05$ significant at 95% CI

4.6 ASSOCIATION OF IMMUNOLOGICAL PARAMETERS AND GRAVIDITY OF MOTHERS AND SEX, METHOD OF DELIVERY, TYPE OF FEEDING AND BIRTH WEIGHT OF THE BABIES.

The association of immunological parameters with gravidity, sex, methods of delivery, type of feeding and birth weight are shown in table 4.7.

There was an association between birth weight and NBT reduction and an increase in 1 kg of birth weight resulted in an increase of 0.351% of NBT reduction. IgG and IgM were also found to have an association with birth weight. An increase in 1 kg of birth weight resulted in increase of 0.503 g/l of serum IgG level. Similarly, an increase in 1 kg of birth weight resulted in an increase of 0.371 g/l of serum IgM level.

For complement levels, there was an association between birth weight and C3 and C4 levels. An increase in 1 kg of birth weight resulted in an increase of 0.540 g/l of C3 level and an increase in 1 kg of birth weight also resulted in an increase of 0.641 g/l of C4 level.

For lymphocyte subsets, there was an association among gravidity with CD3%, CD4% and CD16/56 (NK cell)%. An increase in 1 week of gravidity resulted in an increase of 0.262 % of CD3. Similarly, an increase in 1 week of gravidity resulted in an increase of 0.279 % of CD4. For CD16/56 (NK cell) %, a decrease in 1 week in gravidity resulted in an increase of 0.289 % of CD16/56 (NK cell).

There was no association between IgA level, CD8% and CD19 (B cell)% with gravidity, sex, method of delivery, type of feeding and birth weight.

In summary, NBT reduction, IgG, IgM, C3 and C4 levels were found to be associated with birth weight. In addition, CD3%, CD4% and CD16/56 (NK cell)% were associated with gravidity.

Table 4.7 Association of immunological parameters with gravidity, sex, method of delivery, type of feeding and birth weight.

	Association	Coefficients	p value*
NBT (%)	Birth weight	0.351	0.012
IgG (g/l)	Birth weight	0.503	< 0.001
IgM (g/l)	Birth weight	0.371	0.008
C3 (g/l)	Birth weight	0.540	< 0.001
C4 (g/l)	Birth weight	0.641	< 0.001
CD3%	Gravidity	0.262	0.025
CD4%	Gravidity	0.279	0.022
CD16/56 (NK cell)%	Gravidity	-.289	0.015

* Simple linear regression test, $p < 0.05$ significant at 95% CI

5 DISCUSSION

The immune status of preterm babies at birth is incompletely investigated. To the investigator knowledge not any study has reported about the global immune status of preterm babies. This study was performed to determine the immune status of our preterm infants at birth and to associate the immunological parameters such as, NBT reduction, serum immunoglobulin levels, serum complement levels (C3 and C4) and lymphocyte subsets such as CD3%, CD4%, CD8%, CD19 (B cell) % and CD16/56 (NK cell) % with sepsis in neonatal life.

In this pilot study, cord blood from 36 preterm babies delivered at HUSM was analyzed. Cord blood from 36 term babies was also analyzed as control. In both groups, there were more males than females. The majority of babies were born via spontaneous vaginal deliveries and most of them were given breast milk (table 4.1).

The range of gestational ages in the preterm group varied from 29-36 weeks but the distribution of gestational age was much skewed towards the higher

gestational ages. The majority of preterm babies in the preterm group were indeed 34-36 weeks of gestational age. This reflects that the majority of preterm babies born are not of very low gestational age (< 32 weeks). The immunological parameters may be still more different between these extremely preterm babies and the term group. It is also well known that babies of lower gestational age tend to get more infections and as such the correlation between infection and immunological parameters may differ in the extremely preterm babies from the findings in this pilot study.

5.1 COMPARISON OF IMMUNOLOGICAL PARAMETERS IN THE CORD BLOOD OF PRETERM AND TERM BABIES

5.1.1 NEUTROPHIL FUNCTION (NBT REDUCTION)

In this study, we found that although the NBT reduction test in both preterm and term babies were normal, the mean percentage of NBT reduction in preterm was significantly lower than that in term babies.

These findings differ from the findings by Park *et al* (1970) and Humbert *et al* (1969). These authors found that there was an increased NBT reduction in

preterm babies. Some other authors (Dosset *et al*, 1969; Forman and Stiehm, 1969; Anderson *et al* 1979) reported no difference between the NBT reduction in preterm and term babies. One of the later study however (Irmeli et al, 2002) showed that the neutrophil function was lower in preterm babies than in term babies, which was comparable to a finding in our study. The difference in findings may be explained by difference in the method performed, genetic differences among races or by environmental differences in terms of antigen exposure in the antenatal and perinatal period. It is worth noting that all studies showing disagreement with the findings in the current study were performed more than 20 years ago.

5.1.2 SERUM COMPLEMENT LEVELS

In this study we measured C3 and C4 level in preterm and term babies using immunoturbidometry technique. In agreement with Wolach *et al* (1997), we found that serum complement levels were significantly lower in preterm babies than in the term control group. The level of C3 in our study was comparable to previous study (Wolach *et al,* 1997).

Although there are only few data on the complement system in the preterm babies, quantitative and qualitative defects in complement components have been widely reported in full term babies. The level of serum hemolytic activity and the complement components of the classical and alternative pathways of full term newborn infants have been reported to be significantly lower as compared to adult levels (Shapiro *et al*, 1981; Zilow *et al*, 1993). Another study showed that serum complements levels of full term babies were approximately 60 – 100% of normal adult values (Drew *et al*, 1980).

5.1.3 IMMUNOGLOBULIN LEVELS

5.1.3.1 IMMUNOGLOBULIN G (IgG)

When we compared levels of IgG in term and preterm babies, we found that the mean IgG level in preterm babies was significantly lower than in term babies. The level of IgG in both groups did not differ from those obtained in the study by Marissa *et al* (1986). The results reported by Howarth *et al* (1965) for 19 babies with birth weight lower than 2440g also did not differ significantly from those reported here. The IgG level of preterm babies in this study also agreed with the

work by Conway et al (1985). Levels of IgG in preterm babies were directly proportional with gestational age (Rothberg, 1969; Hobbs, 1967)

However, Ballow *et al* (1986) in a study of babies with birth weight lower than 1500g, detected lower IgG values than those reported here. Sasidharan (1988) in a study of 42 infants of very low birth weight with gestational ages ranging from 23 to 31 weeks, detected extremely low levels of IgG. However, these results cannot be compared to those of the present study since the preterms evaluated by them consisted of babies of lower gestational age and belonged to a population of more favourable socioeconomic background than those studied here, a fact that may have reduced the environmental antigenic stimulation.

As for full term babies, serum level of IgG was comparable maternal levels (Kohler, 1966). However, so far normal reference ranges for plasma or serum immunoglobulin values for newborn term and preterm infants have not been reported in Malaysia.

5.1.3.2 IMMUNOGLOBULIN M AND IMMUNOGLOBULIN A (IgM and IgA)

In this study, IgM and IgA levels were lower in preterm than in term babies and the levels were significantly different between these two groups. However, in contrast to this study, a study by Marissa *et al* (1986) showed that the level of IgM was higher in preterm babies than in term babies. This may be due to intrauterine infection (Cederquist *et al*, 1978; Papadatos *et al*, 1969) that can increase the synthesis of IgM.

IgA level was also significantly lower in preterm than in term babies. IgA level was found in all babies in contrast to the study by Marissa *et al* (1986), where it was found in only 10 of 57 samples (17.5%) and a study by Conway *et al*, where it was found in only 3 out of 115 babies (2.6%). This may be due to different method performed and genetic differences in terms of antigen exposure in the antenatal and perinatal period.

5.1.4 LYMPHOCYTE SUBSET

The three-colour immunofluorescence reagent (TriTEST) has shown a number of differences between lymphocyte subsets of preterm and term babies. Only CD3%

was significantly different between 2 groups. In contrast, CD4%, CD8%, CD19 (B cell) % and CD16/56 (NK cell) % were not significantly different.

Fleisher *et al* (1975) recommended that both percentage and absolute numbers of cells should be reported. But in our study, due to organizational problems, the absolute counts were not performed.

Thomas and Linch (1983) had measured lymphocyte subset in 37 preterm babies and 20 term babies in a white population and found that there was no significant difference in absolute numbers of lymphocyte subsets between term and preterm appropriate for gestational age infants. In their study, they only measured the CD3, CD4, CD8 and CD19 (B cell) but not the CD16/56 (NK cell). They also measured both percentages and absolute count of these lymphocyte subsets.

In the other study, it was shown that healthy preterm babies differed from full term babies by their higher absolute numbers of CD3 and CD4. These increases correlated with gestational age. An increase in CD19 (B cell) was also documented but no correlation with gestational age could be seen (Pichette *et al*, 1991).

5.2 ASSOCIATION BETWEEN IMMUNOLOGICAL PARAMETERS IN PRETERM BABIES AND SEPSIS

Greater susceptibility to certain types of infection and less favourable outcome during the neonatal period has been documented among preterm infants. Therefore, the second aim of this study was to evaluate correlation, if any, between immunological parameters and sepsis.

5.2.1 FREQUENCY OF SEPSIS

The frequency of infection in the group of preterm babies in this study was very low (11.1%) compared to previous studies done in HUSM (Halder *et al,* 1999). These differences with other studies are most likely due to the difference in gestational age. In this pilot study, most of the babies were 34 – 36 weeks and quite a percentage of them did not require admission to the NICU.

A weakness of this pilot study is also that none of the cases of infection had a positive blood culture. Even though each case classified as sepsis had multiple signs and symptoms of sepsis, no other causes of deterioration were found and

there was a good response to antibiotics, there was no certainty about the presence of infection

In this study, in all the preterm babies, the infection was thought to affect predominantly the respiratory system. The higher frequency of infections in the lower respiratory airways of preterm babies has been demonstrated by several studies (Ballow *et al*, 1986; Douglas and Mogford, 1953).

In our study, none of the term babies had infection within 2 weeks of follow-up. In Malaysia, the overall reported rate of neonatal sepsis was 5 – 10% (Boo and Chor, 1994). This discrepancy may be due to small sample size in our study.

5.2.2 ASSOCIATION BETWEEN IMMUNOLOGICAL PARAMETER IN PRETERM BABIES AND SEPSIS

Using univariate analysis, we found that only neutrophil function (NBT reduction) and IgA level were associated with occurrence of sepsis in preterm babies. IgG level, IgM level, complement levels and lymphocyte subsets were

not associated with occurrence of sepsis. However, these data may be affected by the limited number of babies with sepsis.

In literature, only association of IgG level and sepsis has been reported but not the other parameters. There was no previous study done to compare our findings on IgA level, IgM level, complement levels and lymphocyte subsets.

If in a larger follow-up study, these results of a lower NBT reduction in the cord blood of babies developing sepsis, preventive intervention could be considered for those preterm babies with very low NBT. Few studies have reported attempts to transfuse PMN's to babies with established sepsis. Three of five groups of researchers found that patients' survival had increased after transfusion of PMNs (Cairo *et al*, 1988; Laurent *et al*, 1981; Christensen *et al*, 1982). Two other groups showed no change of patient survival (Baley *et al*, 1987; Wheeler *et al*, 1987). No study has been described about the role of PMNs transfusion in preventing neonatal sepsis. However, abnormalities in PMNs at birth may indicate a potential role of buffy coat PMNs transfusion as a prophylaxis for neonatal sepsis.

For IgG level, only few researchers tried to determine the definite association of IgG level and sepsis. Our study was consistent with a study done by Conway *et*

al (1985) who found that there was no definite association between IgG concentration and the incidence of presumed or proven infection.

Few researchers believed that the low concentration of IgG level was responsible for increased risk of infection. The administration of intravenous IgG (IVIG) to preterm babies has been studied extensively. Numerous descriptive review articles, commentaries and editorials on the use of IVIG in neonates have been published. Work in this area to date has produced conflicting results. Lacy and Ohlsson (1995) concluded that routine administration of IVIG to preterm babies to prevent infection was not recommended. The results of a Canadian multidisciplinary consensus-building initiative (Consensus, 1997), has been published and the use of IVIG for prophylaxis of neonatal nosocomial infection was considered to be inappropriate.

Ohlsson and Lacy (2003) concluded that IVIG administration results in a 3% reduction in sepsis and 4% reduction in any serious infection. Most importantly, IVIG administration did not have any significant effect on mortality from any cause or from infections. Prophylactic use of IVIG was not associated with any short-term serious side effects. From a clinical perspective a 3-4% reduction in nosocomial infections without a reduction in mortality or other important clinical outcomes is of marginal importance.

In summary, neutrophil function (NBT reduction) and IgA level were associated with occurrence of sepsis in preterm babies. IgG level, IgM level, complement levels and lymphocyte subsets were not associated with occurrence of sepsis. However, this may be due to limited numbers of babies with sepsis.

5.3 EFFECT OF ANTENATAL DEXAMETHASONE ON IMMUNOLOGICAL PARAMETERS IN PRETERM BABIES.

In this study, immunological parameters in babies born to mothers receiving IM dexamethasone were not significantly different with those born to mothers not receiving IM dexamethasone. There was no previous study looking into this matter.

Antenatal corticosteroid was first introduced in 1972 to enhance fetal lung maturity. Corticosteroids function as "switch" triggers in biology. One effect is to "turn off" cell proliferation and initiate maturation of the cell function. It is this mechanism that is believed to be responsible for the beneficial effects of

antenatal steroid on foetal pulmonary development. The agents stimulate the maturation of alveolar type 2 cells surfactant production and trigger architectural maturation of the foetal lung, thereby reducing the thickness of the alveolar membrane as well as the diffusion distance between alveolar gas and pulmonary capillary (Ballard, 1986).

A meta-analysis (Crowley et al, 1990) concluded that antenatal corticosteroid administration prior to anticipated preterm delivery is associated with substantial reduction in the incidence of early neonatal death, as well as in respiratory distress syndrome, intraventricular hemorrhage, and necrotizing enterocolitis.

5.4 ASSOCIATION OF IMMUNOLOGICAL PARAMETERS AND GRAVIDITY OF MOTHERS AND SEX, METHOD OF DELIVERY, TYPE OF FEEDING AND BIRTH WEIGHT OF THE BABIES.

In this study, NBT reduction, IgG, IgM, C3 and C4 levels were found to be associated with birth weight. In addition, CD3%, CD4% and CD16/56 (NK cell)% were associated with gravidity. There was no association between IgA level, CD8% and CD19 (B cell)% with gravidity, sex, method of delivery, type of feeding and birth weight.

Conway *et al* (1985) reported that IgG but not IgM or IgA level was affected by birth weight. For complement levels, Wolach *et al* (1997) found that there was no significant association of C3 or C4 level with birth weight, method of delivery or gender. For lymphocyte subsets, no study has been done to determine the association of the lymphocyte subsets with gravidity, sex, birth weight, type of feeding or method of delivery.

6 LIMITATIONS AND RECOMMENDATIONS

Most of the limitations of this study have been discussed in the previous chapter. Just to recapitulate, the most important limitations of this study include the following:

1 Small sample size

a. This study was designed as a pilot study with a limited number of subjects. This limits to a certain extent the power of the study to demonstrate differences in a significant way but a pilot study is still useful in showing trends and determining the focus and the samples required for a subsequent bigger study.

b The small sample size made it also not suitable to perform a multivariate logistic regression analysis. In a bigger study, this analysis is still necessary to show whether the significant differences among the preterm and term babies are mainly due to difference in gestational age or due to confounders.

2 Skewed distribution of gestational age in the preterm group

There was a huge majority of the preterm babies having gestational ages between 34 to 36 weeks of gestational age. This made the incidence of infection low and its correlation with the cord blood immunological parameters more difficult. In a larger follow-up study, care should be taken to have a wide range of gestational ages including a larger proportion of babies with gestational ages of less than 32 weeks and even less than 28 weeks.

3 Due to organizational problems, the cell counts in the cord blood were not included in this study. This should be certainty included in a bigger follow-up study, so interpretation of data on lymphocyte subsets become more meaningful.

7 CONCLUSION

In conclusion, this study showed the most immunological parameters tested were lower in preterm babies than in term babies. Complement factors, C3 and C4, IgG, IgA and IgM all had significantly lower serum levels in preterm than in term babies. The NBT reduction was also found to be significantly lower in the preterm group. Among the lymphocyte subsets, CD3% (but not CD4%, CD8%, CD19 (B cell) % and CD16/56 (NK cell) %) was found to be significantly lower in preterm than in term babies.

The small number of cases of sepsis made interpretation of data in correlation to septicaemia difficult. After univariate analysis, there was a significant association between the occurrence of infection during the first two weeks of life and lower NBT reduction as well as lower serum IgA levels.

From this pilot study, it looks like antenatal steroids are not affecting the immunological parameters significantly but again sample size was small to definite conclusions.

It is recommended that a follow-up study with much larger samples and by passing the limitations mentioned should be performed. Such a study could show more definitely, which of the parameters are different between preterm and term babies and which parameters are associated with the occurrence of sepsis in the first two weeks of life. A larger study may also confirm whether antenatal steroids have any effect on the immunological parameters in the cord blood.

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Appendix 1

Consent Form

Borang maklumat dan keizinan pesakit

Status imun dalam bayi yang tidak cukup bulan di hospital USM dan kaitannya dengan infeksi.

Pengenalan

Anda dipelawa untuk menyertai satu kajian penyelidikan secara sukarela dimana darah dari uri diperlukan sebanyak 5 ml. untuk membuat ujian-ujian imunologi (immunoglobulin serum, ujian fungsi neutropil, paras complement C3 dan C4 dan subset limfosit) untuk menentukan status imun bayi anda yang tidak cukup bulan dan juga yang cukup bulan. Sebelum anda bersetuju untuk menyertai kajian penyelidikan ini, adalah penting anda membaca dan memahami borang ini. Ia menghuraikan tujuan, prosedur, manfaat, risiko dan kerahsiaan kajian ini. Sekiranya anda menyertai kajian ini, anda akan menerima satu salinan borang ini untuk disimpan sebagai rekod anda.

Tujuan

Kajian ini bertujuan untuk menentukan parameter-parameter imunologi dalam darah dari uri bayi-bayi yang tidak cukup bulan. Juga untuk menentukan keberkesanan parameter-parameter tersebut dalam meramalkan infeksi semasa hayat neonatal.

Kelayakan Penyertaan

Doktor yang bertanggungjawab dalam kajian ini telah membincangkan kelayakan untuk menyertai kajian ini dengan anda. Adalah penting anda berterus terang dengan doktor tersebut tentang sejarah kesihatan anda semasa mengandung.

Manfaat yang mungkin

Manfaat yang didapati dari kajian ini adalah untuk mencari jalan penyelesaian dengan masalah infeksi yang dialami terutamanya oleh bayi-bayi yang tidak cukup bulan. Mungkin dengan kajian ini kita boleh mengubati bayi-bayi yang tidak cukup bulan ini sebelum terjadinya infeksi.
Jika ada sebarang keputusan yang tidak normal anda dan juga doktor yang merawat bayi anda akan diberitahu.

Kerahsiaan

Maklumat perubatan anda akan dirahsiakan oleh doktor dan kakitangan kajian dan tidak didedahkan secara umum melainkan jika ia dikehendakki oleh undang-undang.
Dengan menandatangani borang persetujuan ini, anda membenarkan penelitian rekod dan penyimpanan maklumat dijalankan.

Pertanyaan

Sebarang pertanyaan mengenai kajian ini, puan-puan bolehlah berhubung terus dengan Dr. Che Maraina Che Hussin di nombor talipon 09/7663000 talian 4343.

Tandatangan

Untuk dimasukkan ke dalam kajian ini, anda mesti menandatangani serta menarikhkan halaman tandatangan (**Lampiran 1**). Sekiranya anda tidak berkemampuan untuk menandatangani borang sebelum kelahiran bayi, pihak kami akan mendapatkan persetujuan secara lisan dahulu dan selepas kelahiran, bolehlah anda menandatangani borang berkenaan.

Keperluan untuk menyertai kajian ini adalah:

- Kandungan anda adalah kurang daripada 37 minggu.

Anda tidak boleh menyertai kajian ini sekiranya:

- Kandungan anda mengalami infeksi semasa lahir.
- Kandungan anda mengalami kecacatan anggota badan.
- Kandungan anda mengalami membran ruptur yang tidak matang.

Darah dari uri diperlukan hanya sekali iaitu semasa lahir sebanyak 5 ml

Prosedur kajian

Sekiranya anda setuju untuk menyertai kajian ini, selepas bayi anda lahir, doktor akan mengambil darah sebanyak 5 ml dari uri. Darah ini akan digunakan untuk menjalankan ujian-ujian imunologi yang dijelaskan seperti di atas. Dengan keputusan yang diperolehi nanti, kita akan memantau bayi anda semasa dalam wad samada akan memperolehi infeksi atau tidak. Selepas ujian dilakukan, darah atau serum akan dilupuskan.

Risiko

Tiada risiko dalam kajian ini.

Penyertaan dalam kajian

Penyertaan anda dalam kajian ini adalah secara sukarela. Anda boleh menolak penyertaan dalam kajian ini tanpa sebarang hukuman.
Penyertaan anda mungkin juga diberhentikan oleh doktor kajian sekiranya anda didapati tidak berkelayakan menyertai kajian ini.

Borang Maklumat dan Keizinan Pesakit
Lampiran 1
Halaman Tandatangan

Untuk menyertai kajian ini, anda atau wakil sah anda mesti menandatangani mukasurat ini.

Dengan menandatangani mukasurat ini, saya mengesahkan yang berikut:

- ○ Saya telah membaca semua maklumat dalam Borang Maklumat dan Keizinan Pesakit ini, dan saya telahpun diberi masa yang mencukupi untuk mempertimbangkan maklumat tersebut.
- ○ Semua soalan-soalan saya telah dijawab dengan memuaskan
- ○ Saya, secara sukarela, bersetuju menyertai kajian penyelidikan ini, mematuhi segala prosedur kajian dan memberi maklumat yang diperlukan kepada doktor, para jururawat dan juga kakitangan lain yang berkaitan apabila diminta.
- ○ Saya boleh menamatkan penyertaan saya dalam kajian ini pada bila-bila masa.
- ○ Saya telahpun menerima satu salinan Borang Maklumat dan Keizinan Pesakit untuk simpanan peribadi saya.

Nama Pesakit (Ditera atau Ditaip)	Nama Singkatan dan Nombor Pesakit
No. Kad Pengenalan Pesakit (baru)	(lama)
Tandatangan Pesakit atau Wakil Sah	Tarikh (ddMMyy) (tambahkan masa jika sesuai)
Nama Individu yang Mengendalikan Perbincangan Keizinan (Ditera atau Ditaip)	
Tandatangan Individu Mengendalikan Perbincangan Keizinan	Tarikh (ddMMyy)
Nama Saksi dan Tanda Tangan	Tarikh (ddMMyy)

Appendix 2

Example of result of lymphocyte subset using Immunophenotyping technique

Sample Name: Yusnita Haizan Yusoff
Sample ID: A 191077
Case Number: 2
Panel Name: tritest

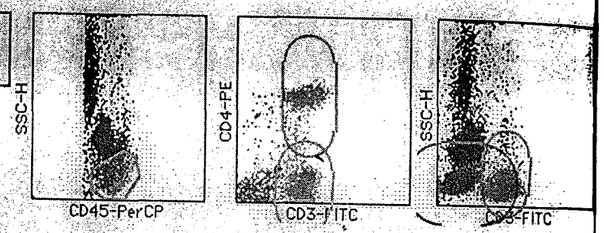
Date Acquired: Tue, Feb 10, 2004 9:24 AM
Date Analyzed: Tue, Feb 10, 2004
Ref. Range Type: BD

CD3/CD4/CD45

Reagent Lot ID: 00000 Events Acquired: 15000

Data Set [1] Data File: Yusnita Haizan Yusoff02.01
Attr Def File: 3/4/45 Tri v2.0

Lymph Events	10247
CD3+ %Lymph	55
CD3+CD4+ %Lymph	33

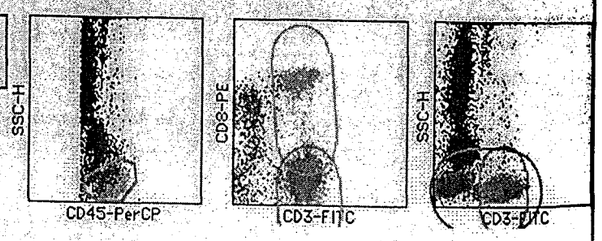


CD3/CD8/CD45

Reagent Lot ID: 00000 Events Acquired: 16500

Data Set [1] Data File: Yusnita Haizan Yusoff02.02
Attr Def File: 3/8/45 Tri v2.0

Lymph Events	9507
CD3+ %Lymph	66
CD3+CD8+ %Lymph	24

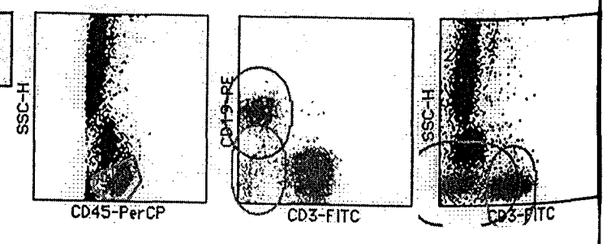


CD3/CD19/CD45

Reagent Lot ID: 00000 Events Acquired: 16500

Data Set [1] Data File: Yusnita Haizan Yusoff02.03
Attr Def File: 3/19/45 Tri v2.0

Lymph Events	8857
CD3+ %Lymph	65
CD19+ %Lymph	21



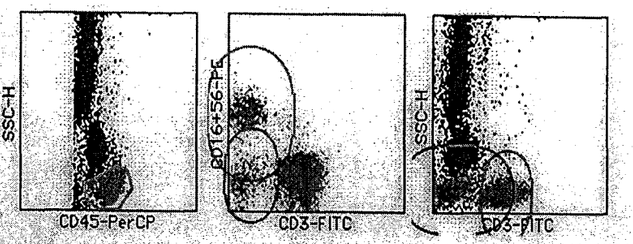
Sample ID: Yusnita Haizan Yusoff
Case Number: A 191077
Panel Name: 2

CD3/CD16+56/CD45

Reagent Lot ID: 00000 Events Acquired: 16500

Data Set [1] Data File: Yusnita Haizan Yusoff02.04
Attr Def File: 3/16+56/45 Tri v2.1

Lymph Events	9251
CD3+ %Lymph	66
CD16+56+ %Lymph	11



Multi-tube QC

Lymphosum:	95.0
CD3 % Lymph Difference:	11
T Helper/Suppressor Ratio:	1.39

usm

MultiSET™ Physician Report

Director: Prof. Normah
Operator: malisa

Software: MultiSET V1.1.1
Cytometer: FACSCalibur (#E2019)

Sample Name: Yusnita Halzan Yusoff
Sample ID: A 191077
Case Number: 2
Panel Name: tritest

Date Acquired: Tue, Feb 10, 2004 9:24 AM
Date Analyzed: Tue, Feb 10, 2004
Reference Range Type: BD

Result Name	%/Ratio	Abs Cnt (cells/µL)	Reference Range
T Lymphs % of Lymphs (CD3+/CD45+)	63		55% 84%
T Helper % of Lymphs (CD3+CD4+/CD45+)	33		31% 60%
T Suppressor % of Lymphs (CD3+CD8+/CD45+)	24		13% 41%
B Lymphs % of Lymphs (CD19+/CD45+)	21		6% 25%
NK Lymphs % of Lymphs (CD16+56+/CD45+)	11		5% 27%

Multi-tube QC

Lymphosum:	95.0
CD3 % Lymph Difference:	11
T Helper/Suppressor Ratio:	1.39

Comments:

Laboratory Director:

This is to certify that

Dr. Siti Hajar Haji Hussin (as a Free Paper Presenter)

has participated

"Laboratory Medicine In Asia: Current and Future"

in

The 5th National Congress of the Asian Society of Clinical Pathology and Laboratory Medicine (ASCPaLM)

and

The 1st Joint meeting with Scientific Sessions of the College of Pathologists, Academy Of Medicine Malaysia (CPath-AMM)

and

The 8th International Congress of the Indonesian Association of Clinical Pathologists (PDS PatKlin)

Held from November 30 until December 02, 2004
at SELECTA CONVENTION HALL Medan - Indonesia

We would like to convey our appreciation and gratitude

Medan, 02 December 2004
Chairman of the Congress

Dr. Adi Koesoema Aman, SpPK

SKPB IDI / No. 237 / PB / A.7 / 10 / 2004	
Jakarta, 13 Oktober 2004	
PESERTA DOKTER	10 SKP IDI
PEMBICARA DOKTER	3 SKP IDI

007 – O

COMPARISON OF CORD BLOOD LEVELS OF IMMUNOLOGICAL MARKERS BETWEEN PRETERM AND TERM BABIES

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Background and aim: Preterm babies are at a much higher risk of neonatal sepsis than term babies. Many factors may be responsible for this, including invasive procedures done in preterm babies, feeding modality and maybe most important of all, the deficient host defense in babies. The aim of this study was to compare the cord blood values of the most important immunological parameters between Malay preterm and term neonates
Methods: Cord blood was taken from 36 preterm and 36 term neonates and the following parameters were determined in the immunology lab: Immunoglobulins G, A and M, Complement factors 3 and 4, NBT and lymphocyte subsets. Results were compared using the t-test (parametric variables) or Mann-Whitney U test (non parametric variables)
Results: The preterm babies had significantly lower cord blood levels of IgG, IgA and IgM than the term babies (9.6 versus 14.3 g/l, $p<0.001$; 210 versus.225 mg/l; $p= 0.035$ and 0.100 versus 0.200 g/l respectively). Complement levels C3 and C4 were also significantly lower in preterm than in term babies (0.719 versus 0.511 g/l, $p<0.001$ and 0.077 versus 0.137, $p<0.001$ respectively). NBT was also significantly lower in preterm than in term babies (7.5 versus 12.0%, $p<0.001$) There were no significant differences among the lymphocyte subsets.
Conclusion: Most humoral immunological parameters were significantly lower in the cord blood of preterm babies than in the term babies. NBT was also significantly lower in preterm babies. There was significant difference found for the lymphocyte subsets. These findings were similar as those of studies done in other populations except for the NBT test.

IMMUNE STATUS IN PRETERM BABIES AND ITS ASSOCIATION WITH SEPSIS

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Background: Preterm babies are at a much higher risk of neonatal sepsis than term babies. Many factors may be responsible for this, including the deficient host defense in babies. Several studies have shown reduced levels of immunological parameters in the cord blood of preterm babies. This is the first study aiming to compare immunological parameters in the cord blood between preterm babies developing sepsis and those not developing sepsis

Methodology: In this pilot study, cord blood was taken from 36 preterm neonates and the following parameters were determined in the immunology lab: Immunoglobulin G, A and M, Complement factors 3 and 4, NBT and lymphocyte subsets. All babies were prospectively followed up for the first two weeks after birth for the development of neonatal sepsis. Results were compared using the t-test (parametric variables) or Mann-Whitney U test (non-parametric variables)

Results: Eleven percent of the subjects developed clinical septicaemia. Overall these preterm babies had significantly reduced cord blood levels of IgG, IgA, IgM, complement factors C3 and C4 and reduced NBT, but only IgA levels and NBT reduction were significantly lower in babies with septicaemia during the first two weeks than in those without septicaemia (0.19 vs 0.21 g/l; p value: 0.007 and 3.50% vs 8.00%; p value: 0.017 respectively)

Conclusion: Even though most immunological parameters tested were reduced in the cord blood of preterm babies, only low IgA levels and low NBT reduction were significantly associated with the occurrence of clinical septicaemia in preterm neonates.