

ORIGINAL ARTICLE

COMPARISON BETWEEN IMMUNOLOGICAL MARKERS IN CORD BLOOD OF PRETERM AND TERM BABIES IN HOSPITAL USM

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A cross sectional pilot study using convenient sampling method was conducted to evaluate various immunological parameters in preterm babies and term babies. Cord blood from 36 preterm and 36 term babies was taken and the following parameters were determined: Immunoglobulin G, A and M, Complement 3 and 4 and NBT. The results showed that NBT was significantly reduced in preterm babies compared to term babies (7.5% versus 12.0%; $p=0.001$). The complement levels, C3 (0.5114 versus 0.7192 g/l; $p<0.001$) and C4 (0.07 versus 0.14g/l; $p<0.001$) were significantly lower in preterm babies than in the term babies. The mean IgG level in preterm babies was significantly lower than in term babies (9.5583 versus 14.2806 g/l, $p<0.001$). IgM (0.1 versus 0.2g/l; $p<0.001$) and IgA (0.210 versus 0.225g/l; $p=0.0361$) levels were significantly lower in the preterm than in term babies. In conclusion, we found that NBT reduction, IgG, IgA, IgM, C3 and C4 levels were significantly lower in the preterm compared to term babies.

Key words : Preterm babies, term babies, immunoglobulin, complement, NBT, lymphocyte

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Introduction

Nosocomial septicaemia is among the most feared and common complications faced by the preterm babies (1). Many factors predispose the preterm babies to infection and an immature immune system is thought to be among the most important ones. Neonatal host defences are immunologically immature and therefore contribute substantially to the high incidence of overwhelming sepsis (2).

Several studies have looked at individual components of the immune system in cord blood of preterm babies and found that most components had either a lower serum concentration or function than in term babies (3-15). A study on 64 preterm babies showed that IgG values at birth ranged from 2.6 g/l to 14.4 g/l (3). Multiple regression analysis showed that the concentration of IgG at birth correlated with gestational age and birth weight. IgM levels ranged from 0 – 0.6 g/l and IgA was detectable in only 10 of 57 samples. However, there was no correlation between gestational age or birth weight and the concentration of IgM and IgA at birth.

Another study compared the levels of the classical complement pathway including total haemolytic activity (CH50), C1q, C2 and C3 in the sera of full term babies with those of preterm babies (4). The values of all classical pathway parameters were much lower in preterm babies than in normal full term babies. More recently, it was confirmed that preterm babies had lower complement levels and activity than term babies (5).

Absolute numbers of circulating neutrophils are increased in neonates (both term and preterm) but the reserve or storage pool available in times of challenge is reduced and easily depleted (6, 7). In addition, neonatal neutrophils show a number of functional deficiencies, namely in chemotaxis, phagocytosis and bactericidal killing, particularly when put under stress such as hypoxia or sepsis (8, 9). Studies on the capability of neutrophils to reduce NBT have shown conflicting results with some studies showing normal NBT reduction (10), while others a decreased (11) or increased reduction (12,13).

Most of the above studies were done more than a decade ago. Recent changes in antenatal management such as the use of antenatal corticosteroids and better antenatal follow-up may have changed the immunological profile and well-being of preterm babies at birth. This makes it timely to conduct a study with the aim to determine various immunological parameters in preterm babies and compare the results with those in term babies.

There has been no previous study evaluating simultaneously a multitude of immunological parameters in preterm and term babies. The relationship between the several components of the immune system in preterm babies was also determined.

Materials and Methods

A cross sectional study using a convenient sampling method was conducted in Hospital Universiti Sains Malaysia (HUSM), a tertiary hospital in Kelantan, Malaysia, from April 2003 till June 2004.

Cord blood was taken from 36 preterm and 36 term babies in the labour room and later analyzed in the Immunology Laboratory. Inclusion in the study was based on the convenience of the main investigator (during times when she was working or on call). Preterm birth was defined as birth before 37 weeks of gestation. Term babies were born between 37 to 42 weeks of gestation. The gestational age of an infant was determined by the first day of the last menstrual period or if this was not available by an early antenatal ultrasound.

The newborns were excluded from the study if they were suspected to have an intrauterine or congenital infection. Any congenital abnormalities in the baby or any maternal medical illness also resulted in exclusion from the study. Presence of intrauterine infection or congenital abnormalities was diagnosed, based on history and physical examination.

Five millilitres of cord blood was taken from both preterm and term babies in 2 different bottles for the following investigations: immunoglobulin levels (serum centrifuged from whole blood), complement levels, neutrophil function (heparinised blood).

Determination of immunoglobulin levels and complement levels was performed through immunoturbidometry technique (Orion Diagnostica, Finland) using turbid analyzer. Neutrophil function was tested by using the nitroblue tetrazolium test

(NBT) from Sigma Aldrich (USA).

All data were entered and analyzed by means of statistical software SPSS version 11. Results were compared using the t-test (parametric variables) or Mann-Whitney U test (non parametric variables). Correlation between parameters was determined using Pearson correlation.

The study was approved by the hospital ethics and research committee. Written informed consent was obtained from the parents.

Results

Cord blood samples of 36 preterm and 36 term babies were taken and analyzed. Table 1 shows the sociodemographic characteristics of the subjects. There were more males than females in each group. The sex distribution and the previous obstetric history (gravidity, parity and number of abortions) were very similar in both groups. The mean period of amenorrhoea (POA) of mothers of preterm babies was 34.47 weeks of gestation (ranging 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).

The comparison of immunological parameters between preterm and term babies is shown in tables 2 and 3. Table 2 shows the immunological parameters that were normally distributed and table 3 shows the not normally distributed immunological parameters.

Significant differences in immunological parameters were found between the term and preterm babies. Each IgG, IgA and IgM had lower mean or median serum levels in the preterm group than in the term group. The differences between both groups were most marked for IgG and IgM ($p < 0.001$). For IgA, the difference was less marked but still reached significance ($p = 0.036$). All babies had detectable levels of IgA

Serum C3 and C4 levels were significantly lower in preterm than in term babies ($p < 0.001$). There was a significant difference of NBT reduction between preterm and term babies ($p = 0.001$).

For preterm babies, correlations were sought between parameters. IgG levels correlated well with gestational age ($r = 0.66$, $p < 0.001$), with NBT ($r = 0.45$, $p < 0.001$), with IgM ($r = 0.43$, $p < 0.001$) and with C3 and C4 ($r = 0.68$, $p < 0.001$ and, $r = 0.63$, $p < 0.001$ respectively). The correlation between IgG and IgA was weak and not statistically significant ($r = 0.27$, $p = 0.21$)

Discussion

Various immunological parameters were found to be significantly lower in preterm than in term babies. The levels of IgG in preterm babies were significantly lower than in term babies. This was congruent with findings in previous studies (3, 14, 15). Levels of IgG in preterm babies were directly proportional with gestational age which was also comparable with results of previous studies (16, 17). IgG correlated well with the gestational age of the babies and with other immunological parameters. This may mean that in babies with one immunological disturbance, many more parameters may be disturbed, rendering the effect of single interventions in the postnatal period to prevent or treat sepsis less effective.

The present study showed that the IgM levels were significantly lower in preterm than in term babies. This differs from a previous study by Marisa et al (14) which showed higher levels of IgM in preterm than in term babies.

IgA levels were also significantly lower in preterm than in term babies. IgA was found in all babies in contrast to the study by Marissa et al (14), where it was found in only 10 of 57 samples (17.5%) and a study by Conway et al (3) that found detectable levels in only 3 out of 115 babies (2.6%). A different method was used for detection of IgA in these studies (single radial immunodiffusion technique).

Complement factor C3 and C4 were significantly lower in preterm than in term babies. This was comparable with the findings of one previous study (5).

With regard to neutrophil function, the NBT reduction test in both preterm and term babies in the present study were normal but the mean percentage of NBT reduction in preterm babies was significantly lower than that in term babies. Park et al (12) and Humbert et al (13) found, contrary to this, that there was an increased NBT reduction in preterm babies. Other authors (18, 19, 20) reported no difference between the NBT reduction in preterm and term babies. However, a more recent study (11) reported that the neutrophil function was lower in preterm babies than in term babies, which was comparable to the finding in our study.

A major weakness of this study is that for technical reasons absolute counts of neutrophils were not available. NBT test is testing function and can be interpreted independently of the counts.

In conclusion, this study showed that preterm babies were immunocompromised when compared

to term babies. Immunological parameters were significantly lower in preterm than in term babies. A statistically strong correlation between the level of IgG and other immunological parameters may suggest that prematurity affects different immunological parameters to a similar degree.

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