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Polymorph Function in Severely Asphyxiated Newborn Infants

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Summary

Ogala, WN, Audu LI, Gupta R, Umar LW. Polymorph Function in Severely Asphyxiated Newborn Infants. *Nigerian Journal of Paediatrics* **1997; 24:52.** Neutrophil intracellular killing ability and neutrophil chemotaxis were determined in ten severely asphyxiated newborn babies (Apgar score < 3 at 5 minutes) after resuscitation and admission into the special care baby unit. Ten healthy normal newborn babies matched for gestational age, sex and methods of delivery, were similarly studied as controls. Viable bacteria count before and after a three-hour incubation was performed, using the most probable number table. The chemotactic index was determined by comparing polymorph migration towards a chemoattractant with polymorph migration towards a control medium. The bactericidal activity was lower ($P < 0.05$) in asphyxiated infants than in the controls. Similarly, the chemotactic index for the asphyxiated infants was lower than that of controls, although the difference was not significant. It is concluded that severe asphyxia results in general depression of polymorph function.

Introduction

INCREASED susceptibility of the newborn infant to infection is a function of the relative immaturity of its immune system. Specifically, defects in polymorphonuclear function and certain serum factors have been suggested.[1] Various forms of perinatal stress such as prematurity, hyaline membrane disease, meconium aspiration syndrome and asphyxia[2] are associated with increased neonatal susceptibility to infection. Neonatal septicaemia is a risk factor for mortality in asphyxiated babies,[3] while invasive resuscitative measures instituted for severe asphyxia may facilitate the entry of bacteria into the baby. The purpose of the present communication is to report our findings on the investigations of the role of polymorphs in neonatal susceptibility to infection following perinatal asphyxia.

Several methods have been used to study polymorph function in normal and stressed newborn infants. These include nitroblue tetrazolium dye reduction test (NBT),[4] hexose monophosphate shunt activity (HMPS)[1] and chemiluminescence.[5] These methods are cumbersome and time-consuming. Therefore, in the present study, two aspects of polymorph function namely, chemotaxis and intracellular killing were investigated in severely asphyxiated and control infants, using relatively simple and quick methods.[6,7]

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Patients and Methods

Ten severely asphyxiated (Apgar score < 3 at 5 min) term newborn infants admitted into the special care baby unit (SCBU), Ahmadu Bello University Teaching Hospital, Zaria, were studied. They were clinically assessed for gestational age, using the method of Dubowitz *et al*,[8] and their birthweights, sex and mode of delivery were recorded at birth. Babies delivered of diabetic mothers as well as those with history of maternal genital infection, prolonged rupture of membranes, or prolonged labour were excluded from the study. Ten healthy babies (with Apgar score > 7) matched for gestational age, birth weight, sex and mode of delivery were randomly selected as controls. Informed

verbal consent was obtained from the parents or relations before inclusion in the study.

Five millilitres of venous blood was freely withdrawn from each baby soon after resuscitation and admission into the SCBU. The blood was immediately taken to the laboratory for the assays. Total white blood cell and differential counts were performed on all the blood samples by the routine methods used in the haematology laboratory of our hospital. The method described by Philips *et al*,[6] was used to determine neutrophil intracellular killing ability. For this purpose, 200 microlitre (200ul) of neutrophil suspension containing 2 x 10^7 nucleated cells per ml, 150ul of bacterial suspension (*Staphylococcus aureus)*, 50ul of pooled human serum and 600ul of tissue culture-199, were mixed together in a sterile centrifuge tube and left for 10 minutes at 37°C (pre-incubation). The mixture was then re-suspended in 1ml of medium-199 containing penicillin (100 i.u/ml) and streptomycin (100ug/ml). An aliquot of the neutrophil suspension (t_0) was removed, while the remainder was incubated at 37°C for three hours to allow for intracelular killing, after which a second aliquot (t_3) was removed. The aliquots (t_0 and t_3) were then separately washed and the neutrophils were disrupted by sonication and inoculated in a downward dilution in a microtitre tray containing nutrient broth. The number of viable bacteria released was counted for t_0 and t_3, using the most probable number table of Halvorson and Ziegler as described by Philips *et al.*[6] The bacterial killing index (BKI) was then calculated as

$$BKI = \frac{\text{Viable bacteria count after incubation } (t_3)}{\text{Viable bacteria count before incubation } (t_0)}$$

This computational technique defines an inverse relationship for BKI value and bactericidal activity. Chemotaxis was assessed using the method of Nelson *et al.*[7] The directed chemotaxis of neutrophils on agarose towards an antigen (zymosan activated serum) (A) and the random migration of neutrophils towards a non-chemoattractant control medum-199 (B) were measured and the chemotactic index (CI) was calculated as follows: CI = A/B. Analysis of data obtained was carried out, using the Student's 't' test.

Results

Table I shows the relevant features of the 20 infants that were studied. The mean age at which blood was withdrawn from the asphyxiated infants (11.4 ± 6.2 hours) was not significantly different from the 10.0 ± 8.9 hours in the controls ($p > 0.1$). Similarly, the mean birthweight, gestational age and sex ratio were similar for the two groups of babies. However, the mean Apgar score of 2.2 ± 0.9 at five minutes in the asphyxiated infants was lower than the 8.0 ± 1.2 in the controls ($p < 0.001$).

Table I

Relevant Features in 10 Asphyxiated and 10 Control Infants

Feature	*Infants*		*P value*
	Asphyxiated	*Control*	
Age (hr) at blood withdrawal	11.4 ± 6.2	10.0 ± 8.9	>0.05
Birthweight (g)	2590.0 ± 538.0	2780.0 ± 480.0	>0.05
Gestational age (weeks)	37.0 ± 0.7	37.5 ± 1.7	>0.1
Sex ratio (M:F)	1.5:1	1.5:1	>0.1
Apgar score at 5 minutes	2.2 ± 0.9	8.0 ± 1.2	<0.001

The laboratory investigations carried out and summarised in Table II show that the total and differential white cell counts were similar for both the asphyxiated babies and the controls ($p > 0.05$). The bacterial killing index was however, significantly higher ($p < 0.05$) in the asphyxiated infants than in the controls. This result was interpreted to mean that the bacterial activity of the neutrophils in the asphyxiated infants was significantly lower than in the controls. Similarly, the chemotactic index in the asphyxiated infants was lower than that in the controls, although this difference did not reach statistical significance ($p > 0.05$).

Table II

Laboratory Investigations in 10 Asphyxiated and 10 Control Infants

Investigations	*Infants*		*P value*
	Asphyxiated	*Control*	
Total white cell count (x 10^9/L)	7.83 ± 3.75	7.84 ± 3.4	>0.1
Neutrophil count (x 10^9/L)	2.03 ± 0.96	3.61 ± 1.54	>0.05
BKI	2.74 ± 1.98	1.08 ± 0.81	<0.05
CI	2.37 ± 0.49	3.01 ± 0.86	>0.05

BKI = Bacterial killing index
CI = Chemotactic index

Discussion

Prematurity as well as the stress of normal labour are associated with defects in polymorphonuclear leukocyte function.[9 10] The babies in the present study were products of full term gestation, while both the asphyxiated and the control infants were matched for mode of delivery to minimise the confounding effect of the latter on neutrophil function. For the same reason, babies with risk factors for early neonatal infection (prolonged rupture of membranes, prolonged labour and maternal genital infection) were excluded from the study.

The absence of any significant difference in the total and differential white cell counts for the asphyxiated and control infants in our series, is similar to the findings of Anderson, Pickering and Peigin[4] and Stoener *et al.*[1] In contrast to this however, increased polymorph count which has been reported to be associated with asphyxia[10] and other stressful conditions,[11] was not observed in the present study. The reason for this difference in polymorph response to stress in both studies is not clear but could be related to the nature and severity of the underlying stress in each study.

Our study has shown that the polymorph bactericidal activity was significantly depressed in asphyxiated infants and this finding could have resulted partly from the release into circulation, of immature, supposedly less functionally active polymorphs as occurs in conditions of stress.[1 10] The process of polymorph intracellular bacterial killing involves increased oxygen consumption;[12] therefore, hypoxic states will adversely affect this activity. It is therefore, postulated that severe hypoxia resulting from severe asphyxia disrupts the cellular oxidative metabolism central to polymorph bactericidal activity in addition to the release of immature polymorphs. The bacterial killing index as described in the present study strongly indicates that decreased intracellular killing is one of the characteristics of the defects in polymorph function in perinatal asphyxia. The study also showed that there may be a depression in polymorph chemotaxis in asphyxiated infants. Subject to further studies and confirmation of our results, it is concluded that a major effect of severe neonatal asphyxia is depression of polymorph function. Thus, continued high index of suspicion of infection and therefore, infection work-up in all severely asphyxiated newborn infants should be the watchwords so as to enable prompt administration of antibiotics.

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