Curr Pediatr Res 2013; 18 (1): 15-17 *ISSN 0971-9032*
http://www.pediatricresearch.info

Assessment of DNA damage in term neonates with sepsis by comet assay.

Priyadharshini NA, Parkash Chand, VishnuBhat B*, Ramachandra Rao K.

Departments of Anatomy and Paediatrics*, JIPMER Puducherry- 605006, India.

Abstract

Infection is one of the most important cause of neonatal morbidity and mortality. This case control study was undertaken to assess free radical induced DNA damage in babies with neonatal sepsis using single cell gel electrophoresis (Comet assay). Thirty five term babies with sepsis and equal number of age and gender matched controls were included in this study. Mean comet length (125.87 ± 42.95µm Vs 66.62 ± 11.62µm) and tail length (60.05 ± 48.80µm Vs 5.99 ± 4.63µm) were significantly higher (p <0.001) among cases when compared with controls. Similarly percentage of DNA in tail (19.66 ± 10.35 Vs 6.32 ± 2.39) was higher among cases. DNA damage represented as tail length (74.51 ± 49.41 Vs 23.88 ± 21.24µm) was significantly higher (p<0.05) among culture positive neonates when compared with probable sepsis cases (sepsis screening tests positive). Thus there is increased DNA damage among babies with neonatal sepsis and the DNA damage is higher among babies with severe infection.

Keywords: Neonatal sepsis, Comet assay , Free radical injury, DNA damage

Accepted October 13 2013

Introduction

Neonates account for 40% of death under the age of 5 years world-wide. In India though there was decline in neonatal mortality, still it remains very high in comparison to developed country. The primary causes are severe infection, prematurity and birth asphyxia [1,2]. The incidence of neonatal mortality in India is 34 per 1000 live birth [3]. Neonatal sepsis is a clinical syndrome of bacteraemia characterized by systemic signs in the first four week of life. Among the various organisms responsible for neonatal sepsis, klebsiella pneumonia followed by coagulase negative staphylococcus aureus are most common [4]. Endotoxins or exotoxins released by the organism, undergo sequential events to trigger the release of inflammatory mediators mainly NFķB. This complex syndrome is encompassed with the state of redox imbalance produced by dysregulated immune-inflammatory mediators [5]. The equilibrium between the free radical generation and anti oxidant defence is altered in neonatal sepsis and it tilts towards increased free radical generation .Free radical causes damage to DNA. The present study was conducted to assess the DNA damage in neonatal septicaemia.

Material and methods

This study was carried out in the cytogenetics unit of department of Anatomy in collaboration with department of paediatrics from November 2011 to April 2013. The study was approved by institute research council and human ethical committee. Term neonates who were clinically diagnosed and positive for atleast two septic screening tests, were grouped as cases. Septic screening included increased µ-erythrocyte sedimentation rate (µESR), C-reactive protein (CRP) and band cell count [6]. Depending on the blood culture, the cases were sub grouped as confirmed cases with positive blood culture and probable cases with only septic screening positive. Age and gender matched healthy babies were chosen as controls. Babies with birth asphyxia, low birth weight, preterm and congenital malformations were excluded, as these factors would also cause DNA damage.

Assessment of DNA damage was done by Single cell gel electrophoresis (comet assay). Blood samples were collected before starting antibiotics to avoid drug induced DNA damage. Lymphocytes were separated by adding the whole blood to equal volume of histopaque and subjecting them to centrifugation. Lymphocytes were sandwiched between agarose gel layer, followed by electrophoresis, neutralisation and fixation. Later the slides were stained and the comets visualized using Olympus BX53 microscope under 20X magnification. Comet parameters like comet length, Head diameter, tail length, percentage of DNA in head and percentage of DNA in tail were measured using comet score software [7]. Results were analysed using independent student T test with SPSS

software version19. P value <0.05 was taken as significant.

Results

Base line parameters like age, gender and weight were comparable between cases and controls. The mean comet length among cases was 125.87 µm and in controls 66.62µm (p<0.001). The mean tail length among cases was 60.05µm and was significantly higher than the controls. Similarly the percentage of DNA in tail among cases was 19.66 and significantly higher than in controls 6.32 (p<0.001). The tail length of confirmed cases was 74.51 µm and it was higher than probable cases (p<0.05).

Table 1. *Mean comet parameters among cases and controls*

Comet parameters	**Cases (n=35) Mean ± S.D**	**Controls (n= 35) Mean ± S.D**	**P value**
COMET LENGTH (µm)	125.87 ± 42.95	66.62 ±11.62	< 0.001
TAIL LENGTH (µm)	60.05 ± 48.80	5.99± 4.63	<0.001
Percentage of DNA in tail	19.66 ± 10.35	6.32 ± 2.39	<0.001
Percentage of DNA in head	80.33± 10.35	93.67± 2.39	<0.001

Table 2. Mean comet parameters among confirmed and probable cases

Comet parameters	**Confirmed cases (n=25) Mean ± S.D**	**Probable cases n= 10) Mean ± S.D**	**P value**
Comet length (µm)	139.53 ± 43.38	91.74± 11.75	> 0.05
Tail length (µm)	74.51 ± 49.41	23.88 ± 21.24	**< 0.05**
Percentage of DNA in tail	22.19 ± 9.56	13.33 ± 9.95	> 0.05
Percentage of DNA in head	77.08 ± 9.56	86.66 ± 9.95	> 0.05

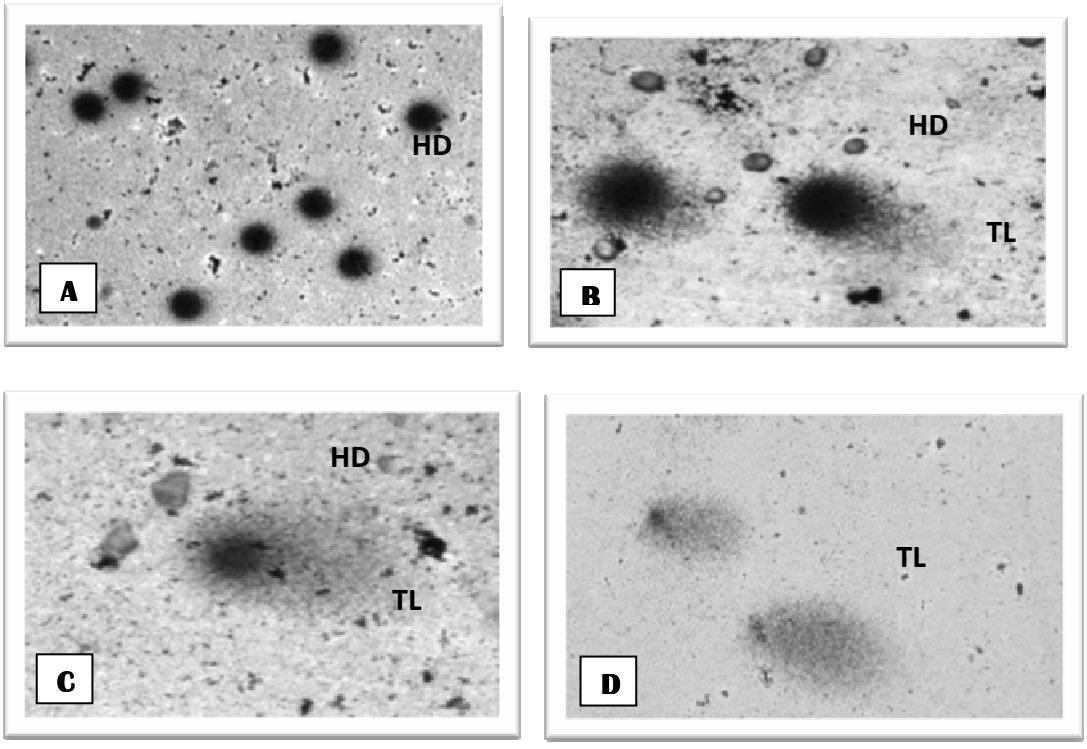

Figure 1. *Images of silver stained comets. A - Controls, B - Probable cases, C&D – Confirmed cases (HD - Head diameter, TL - Tail length)*

Discussion

Infection is one of the major causes of neonatal mortality in developing country like India. To start with, it is an infection but may progress to sepsis, septic shock and even multiorgan dysfunction. As there are only sparse Indian data regarding free radical induced DNA damage in neonatal sepsis, this study has been carried out.We studied the extent of DNA damage and its association with the organism causing it.

Among the comet parameters, Tail length and percentage of DNA in tail are the sensitive indicator of DNA damage. In our study we found that, both of these parameters were significantly increased in cases when compared with controls. Batra et al studied the level of ROS markers in septicaemic babies. our findings were consistent with that of Batra et al [8].

The severity of DNA damage correlated with severity of sepsis and culture positivity. Based on tail length and percentage of DNA in tail, severity of damage is categorised into mild, moderate and severe. Tail length which is directly proportional to damaged DNA was (74.51µm) significantly high ($p<0.05$) among confirmed cases of neonatal sepsis. Inflammatory mediators released in response to toxins cause free radical injury, which explicated as DNA damage. So severity of DNA damage is directly proportional to the severity of the disease.

Carvalho et al studied whether the comet assay could be used as a diagnostic tool for sepsis, but the study was done among proven and probable cases of sepsis [9]. Though there was DNA damage, but significant difference between the two groups was not made out. In the present study DNA damage is compared between cases and matched healthy controls and the parameters indicative of significant DNA damage among babies with neonatal sepsis.

Klebsiella pneumoniae and Staphylococcus aureus are the two most common organisms isolated in the indian tertiary setup. The pattern of organisms causing sepsis also differs from place to place and can change in the same place over a period of time. Overall, in developing countries like india, sepsis due to Gram negative organisms predominate over gram positive.

Ameen turki et al observed in their study that lipid peroxidation as a result of reactive oxygen species production play a significant role in pathogenesis of multiple organ failure and septic shock associated with neonatal sepsis. And the marker of lipid peroxidation, serum MDA (Malondialdehyde) was high in diagnosed cases of sepsis [10].

In our study it was probably the increased level free radicals responsible for DNA damage. The level of DNA damage can predict the severity and clinical outcome of neonatal sepsis. Usage of antioxidant therapy may be considered in sepsis which can combat the raised free radical level.

References

1. Report of the National Neonatal Perinatal Database. Report 2002- 2003. Report No. NNPD network 2005.
2. Bang AT, Bang RA, Baitule SB, Reddy MH, Deshmukh MD. Effect of home-based neonatal care and management of sepsis on neonatal mortality : field trial in rural India. Lancet 1999 http://www.who.int ISBN 978 92 4 156419 9.
3. Zakariya BP, Bhat BV, Harish BN, Arunbabu T, Joseph NM. Neonatal sepsis in a tertiarycare hospital in South India: Bacteriological profile and antibiotic sensitivity pattern. IndianJ Pediatr 2011; 78: 413-417.
4. Macdonald J, Galley HF, Webster NR. Oxidative stress and gene expression in sepsis British Journal of Anaesthesia 2003; 90: 221-232.
5. Sankar MJ, Agarwal R, Deorari AK, Paul VK. Sepsis in the newborn. Indian J Pediatr 2008; 75: 261-266.
6. SinghNP; Mccoy MT; Tice RR; Schneider ELA. Simple technique for quantitation of low levels of DNA damage in individual cells. Exp cell Res 1998; 175 :184-191
7. Batra S, Kumar R, Kapoor AK, Ray G. Alterations in antioxidant status during neonatal sepsis. Ann Trop Paediatr 2000; 20: 27-33.
8. Carvalho NC, de Souza RL, Dal-Pizzol F, de Andrade VM. Comet assay in neonatal sepsis. Indian J Pediatr 2010; 77: 875-877.
9. Ameen T, Majeed MN. Measurement of serum Malondialdehyde (MDA) level as a marker of lipid peroxidation in neonatal sepsis. Thi - Qar Medical Journal 2011; 5: 9-11.

Correspondence to:

Parkash chand
Department of Anatomy
JIPMER, Puducherry -605006
India