Blood Zinc Levels in Children Hospitalized with Severe Pneumonia: A Case Control Study

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Manuscript received: May 21, 2003, Initial review completed: August 11, 2003; Revision accepted:October 27, 2003.

A case control study was conducted in a referral and teaching hospital in North India on children aged 2 months to 5 years, to compare blood zinc levels in 50 cases of severe pneumonia and 50 age, sex and nutritional status matched controls. Mean blood Zinc levels in cases and controls was 376.1 µg/dL ± 225.73 and 538.52 µg/dL ± 228.0 respectively (P value 0.0003). In logistic regression model severe pneumonia was associated with lower blood zinc level, use of biomass fuel and isolation of H. Influenzae from nasopharyngeal swab. Cotrimoxazole resistant S. pneumoniae were isolated from 95% of cases and 41.2% of controls (P = 0.0004). Therefore, the role of zinc in treatment of severe pneumonia should be investigated.

Key words: *Blood zinc, Children, Pneumonia.*

Acute lower respiratory infections (ALRI) predominantly pneumonia cause approximately 4 million deaths every year, accounting for one-third of all childhood deaths in developing countries(1). Various factors have been associated with acute respiratory infectious (ARI) in general and pneumonia in particular. These include, among others, nutritional status(2,3), family characteristics(2,3) and environmental exposures(2,4). Most of environmental risk factors require multisectoral coordination for modification. In contrast, some of the childhood risk factors can be modified by simple interventions like vitamin A or zinc supplementation(5).

Recent works have provided conflicting(6) evidence on the role of zinc against ALRI. The study hypothesis was that there is no difference in blood zinc levels in cases of severe pneumonia as compared to controls.

The primary objective of this study was to compare blood zinc levels in cases of severe pneumonia with age, sex and nutritional status

matched controls was taken. Secondary objectives were to assess antimicrobial resistance in nasopharyngeal isolates of *S. pneumoniae* and *H. influenzae* and to assess the association of use of biomass fuels with severe pneumonia.

Subject and Methods

Study design

This was a hospital based case control study conducted in Department of Pediatrics, King George's Medical College, Lucknow, India between August 2001 to May 2002. Included were children between ages of 2 months to 5 years, admitted in the indoor pediatric ward and satisfying the World Health Organization (WHO) case definition of severe pneumonia(7). Healthy children attending pediatric outdoor for immunization were controls. Informed and written consent of the parents of cases and controls was taken. Excluded were children less than 2 months or more than 5 years, with an associated clinical diagnosis of diarrhea, allergic diseases or asthma, or known zinc supplementation and documented parenteral antibiotic intake prior to indoor admission for current illness.

Sample size

Fifty cases of severe pneumonia and 50 sex, age and nutritional status matched controls were taken. Age was matched within 6 months. For matching nutritional status weight for height "Z" score were used. This sample size was adequate to assess a difference of 100 µg/dL in mean of blood zinc level in cases and controls with a power of 0.8 and an alpha error of 0.05 with a standard deviation/difference ratio of 1 and adjusted R-square of 0.9.

Variables for data collection and definitions

Data was collected on age, sex, anthropometry, family characteristics, environmental exposures and immunization status. Patients were examined clinically. In cases, data on investigations ordered by the treating physician was abstracted. Record of the treatment given and outcome of the disease was also maintained.

Zinc estimation

For this 2 mL of whole blood was collected using 22 gauge steel needle in pre-heparized Eppendoff's tube through venepuncture using aseptic precautions. It was sent to the Industrial Toxicology Research Center, Lucknow for analysis. Samples were stored at –20ºC, 1 mL of blood was mixed with 5 mL of deionized water, glass beads and 2 mL of 1:1 mixture of concentrated HNO_3 and $HCIO_4$. Samples were digested at 120-150ºC in a fuming chamber over hot plate for approximately 2 hours, until a clear solution was obtained which was re-diluted to 5 mL with de-ionized water(8). A sample blank was always prepared with each set of samples in order to control for possible contamination by external zinc. Estimation of zinc levels was done by atomic abosorption spectrophotometry(8) (Varian-Spectra AA 250 plus, Australia).

Nasopharyngeal swab culture

Calcium alginate swabs(9) were used for nasopharyngeal cultures. In the cases of severe pneumonia, swab was taken within 12 hours of hospital admission. In controls, swabs were taken in outpatient department. Skimmed milk Tryptone, Glucose, Glycerine (STGG medium) was used for transport to the laboratory. For antimicrobial sensitivity, disc diffusion technique was used. Mueller-Hinton sheep blood agar was used for *S. pneumoniae* while Haemophilus test medium was used for *H. influenzae*. Zone of inhibition was measured with rulers and zone size interpreted by reference to National Committee for

Clinical Laboratory standards as susceptible intermediate or resistant(10).

Data management

Data was computerized (visual Fox-pro 6.0, data base management software). Statistical analysis was done using statistical software package. (STATA 6.0. 6th edition 1993(11)). Chi-square test was used for categorical and student's t-test for continuous variable and $p < 0.05$ was considered statistically significant. Logistic regression analysis was used to assess the association of severe pneumonia with blood zinc levels and other variables which had univariate association with case-control status.

Results

There were 50 cases, each of severe pneumonia and age, sex and nutritional status matched controls. Seventy four percent of cases and controls were £12 months of age and 26% in each group were between 12 months to 5 years. There were 70% males in cases and controls. Among cases and controls 66% were adequately nourished while 34% were malnourished. Mean weight was 6.06 kg ± 2.54 and 6.18 kg ± 2.25 in cases and controls, respectively ($p = 0.63$). Mean height was 62.14 cm ± 10.53 and 64.6 cm ± 9.33 in cases and controls, respectively ($p = 0.22$).

Use of biomass fuels emerged as a significant risk factor (Odd's ratio = 2.67; 95% CI: 1.19 - 5.95, $p = 0.027$) for cases of severe pneumonia. Immunization status, family type, availability of separate cooking space, maternal education and smoking status of father was not significantly associated with case control status (*Table I*).

Fever and difficulty in breathing (*pasli chalna)* were the most common complaints found in 98% and 96% of cases respectively. All cases had intercostal retractions and increased respiratory rate. Mean respiratory rate, in cases, was 73.32 ± 18.28 per minute (range 60-180). 10% had cyanosis. While duration of symptoms among cases from rural areas was more by 2-3 days when compared to those from urban areas, the differences were not statistically significant (data not shown).

Mean blood zinc levels among cases and controls by place of residence and nutritional status is given in *Table II*.

S. pneumoniae was isolated from naso-

TABLE I–*Family Characteristics and Environment Exposures among Cases and Control.*

Characteristics	Cases (N = 50)		Controls (N = 50)		Odds	95% CI	P value
	Number	% of cases	Number	% of controls	ratio		
Unimmunized	11	22%	8	16%	1.48	0.54 - 4.06	0.44
Urban Residence	31	62%	35	70%	0.69	0.30 - 1.61	0.4
Joint Family	31	62%	36	72%	0.63	0.27 - 1.47	0.29
Separate cooking space	19	38%	17	34%	1.19	0.53 - 2.69	0.68
Biomass fuels other than LPG	32	64%	18	36%	2.67	1.19 - 5.95	0.027
Mother uneducated	19	38%	16	32%	1.3	0.57 - 2.97	0.67
Father non-smoker	39	78%	36	72%	1.38	0.55 - 4.43	0.64

LPG = Liquified Petroleum Gas.

TABLE II–*Blood Zinc Level (µg/dL) among Cases and Controls.*

Blood Zinc Levels	Cases		Controls		P value
	n	mean ± SD	n	mean ± SD	
Over all	50	376.1 ± 225.73	50	538.52 ± 228.0	0.0003
Urban subjects	31	437.1 ± 239.88	35	511.23 ± 223.85	0.19
Rural subjects	19	276.58 ± 161.06	15	602.2 ± 232.5	0.0001
Malnourished subjects	17	308.24 ± 166.03	17	455.88 ± 174.67	0.02
Adequately nourished subjects	33	411.06 ± 246.03*	33	581.09 ± 242.59	0.06

* p = 0.14 on comparing malnourished with adequately nourished cases;
Adequately nourished: WHO 'Z' score ≥ -1;
Malnourished: WHO 'Z' score < -1.

pharynx of 40% of cases and 34% of controls (p = 0.53). *H. influenzae* was isolated from nasopharynx of 32% of cases and 24% of controls (p = 0.50). *Staphylococcus aureus* and Klebsiella species was isolated in 20% and 6% of cases, respectively, and none of the controls.

Multivariate conditional logistic regression analysis was done to assess the association of blood zinc levels with case/ control status controlling for other variables which either had a univariate association with it or were clinically meaningful or could be potentially modified. Lower blood zinc levels (adjusted OR 0.995 (95% CI: 0.993 - 0.998; p = 0.001), use of fuels other than LPG (adjusted OR 3.87 (96% CI: 1.42-10.59; p = 0.008) and nasopharyngeal colonization with *H. influenzae* (adjusted OR 3.77 (95% CI: 1.15-12.43; p = 0.029) were significantly associated with cases of pneumonia.

Discussion

In the present study we found lower blood zinc level of cases when compared to age, sex and nutritional status matched controls. One explanation for lower zinc level in severe pneumonia can be pre-existing zinc deficiency, making the child susceptible to pneumonia due to impaired immunity(5). In addition, respiratory tract infections are also known to result in lower zinc levels(12). A decline in plasma zinc concentration has been reported after a broad range of febrile illnesses(12). It has also been suggested that lowered zinc level is mediated by interleukins and tumor necrosis factor alpha (TNF-*a*) and is a part of predictable set of metabolic reactions to infection or tissue injury known as acute phase reaction(12).

Use of biomass fuels (coal, wood, dung and kerosene) was significantly associated with severe pneumonia. (*Tables I & II*). Previous studies have also associated use of biomass fuels with respiratory tract infections(3,4).

This was a hospital based case-control study. We have not used radiological confirmation as an inclusion criteria for cases as this has not been recommended by the WHO and national ARI control programs. We did not perform blood or lung aspirate cultures to confirm invasive bacterial isolates. Hence we cannot comment on the correlation between nasopharyngeal and invasive isolates in cases of severe pneumonia in our setup.

Key Messages

- Children with severe pneumonia have lower blood zinc levels than age, sex and nutritional status matched controls.
- Use of biomass fuels and nasopharyngeal colonization with *H. influenzae* are significant risk factors for severe pneumonia.
- Cotrimoxazole resistance in 95% isolates of *S. pneumoniae* from cases of severe pneumonia.

We conclude that cases of severe pneumonia have a significantly lower blood zinc level as compared to age, sex and nutritional status matched controls. Role of zinc in the treatment of severe pneumonia should be investigated. Use of biomass fuels must be phased out. Incorporation of vaccination against *H. influenzae* in national ARI program can be considered. Since a high resistance to cotrimoxazole has been found in *S. pneumoniae* and *H. influenzae* in the present study, a surveillance system should be instituted to monitor changes in antimicrobial resistance with time and the pattern of bacterial isolates from the community.

Contributors: SK participated in designing the study, collecting the data and writing the paper. SA was the principal supervisor participating in designing of the study, interpretation of results and supervised the writing of the paper. AJ supervised the micro-biological procedure and results and helped in writing the paper. RCS supervised the zinc estimation and helped in analysis of the results. SA will act as guarantor of the paper.

Funding: None.

Competing interests: None stated.

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