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# Effect of cryptosporidial and giardial diarrhoea on social maturity, intelligence and physical growth in children in a semi-urban slum in south India

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**Abstract**

*Background*: Early childhood diarrhoea is a major cause of infant morbidity and mortality in developing countries. Recurrent and persistent diarrhoea affect growth and cognition in children as young as 6 years.

*Objectives*: To evaluate the effect of early childhood cryptosporidial and giardial diarrhoea on growth and development in children in a semi-urban slum in India. This is the first report of such assessment at 3 years of age.

*Methods*: This study was undertaken on 116 children who were part of an ongoing birth cohort study ($n=452$) of rotaviral and cryptosporidial diarrhoea between June and December 2005. Social quotients (SQ) assessed by the Vineland Social Maturity Scale, intelligence quotients (IQ) assessed by the Seguin Form Board Test, physical growth parameters and sociodemographic data in 84 children with a history of cryptosporidial or giardial diarrhoea were compared with those of 32 without diarrhoea.

*Results*: Children with a past history of giardial diarrhoea showed a trend towards lower SQ ($p=0.09$) and had significantly lower IQ ($p=0.04$) and increased wasting ($p=0.04$). Cryptosporidial diarrhoea was not associated with poor IQ, SQ or physical growth.

*Conclusion*: This study demonstrates the long-term effect of protozoan diarrhoea, especially that caused by giardia, on both intelligence and physical growth in Indian children as early as 3 years of age and re-inforces the need for early detection and prevention of early childhood protozoan diarrhoea.

## Introduction

In developing countries, children below the age of 5 years experience at least three episodes of diarrhoea per year.[1] Although a leading cause of under-5 morbidity and mortality, the actual effects of repeated and chronic diarrhoea on development and future productivity as adults is grossly under-estimated.[2] Recurrent and persistent diarrhoea has been consistently associated with stunting in children. In studies in Brazil and Peru, early childhood diarrhoea, defined as episodes of diarrhoea in the 1st 2 years of life, negatively correlated with tests of cognitive function, verbal fluency and physical fitness and resulted in long-term growth faltering.[3–7] These studies show that the effects of early childhood diarrhoea are more far-reaching than merely causing dehydrating diarrhoeal illness. In these studies, diarrhoea caused by two protozoan parasites, giardia spp. and cryptosporidium spp., was frequently found to be associated with stunting and lower cognitive function scores.[3,4] These

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protozoan parasites are a common cause of symptomatic and asymptomatic enteric infections in children in India,[8,9] the long-term effects of which have yet to be studied.

This study aimed to document the effect on childhood development of early childhood diarrhoea caused by cryptosporidium spp. and giardia spp. by assessing social maturity and intelligence quotient (IQ) as markers of cognitive function as well as physical growth parameters in a cohort of south Indian children at 3 years of age. It is the first such report from India.

## Methods

### *Study design*

The study was a cross-sectional analysis of part of the birth cohort of children.

### *Setting*

Children enrolled in the study were part of a birth cohort of 452 from a semi-urban slum in Vellore, South India[10] in whom diarrhoeal episodes were being followed for a period of 3 years for studies on rotaviral,[11] giardial[12] and cryptosporidial diarrhoea.[13] The study area was three adjacent semi-urban slums in Vellore, Ramnaickanpalayam, Chinnallapuram and Kaspa, with a population of approximately 33,390 and covering an area of 2.2 $km^2$. Each household in the cohort was visited soon after the enrolled child's birth to obtain baseline socio-demographic information including socio-economic status, education of the mother and head of the household, total number of siblings, birth order, maternal age, method of stool disposal and duration of breastfeeding. Assessment of socio-economic status was based on the modified Kuppusamy scale.[14] The children were then visited twice a week by field workers and regularly followed up for all illnesses. A diarrhoeal episode was defined as at least three watery stools a day, preceded and followed by 2 days without diarrhoea.[15] During a diarrheal episode, its duration, the number of stools passed in each 24-hour period, duration of vomiting (if present), number of vomiting episodes in each 24 hours, fever (in °C) and the degree of dehydration were recorded. Faecal samples collected during all episodes of diarrhoea were microscopically examined for cryptosporidium oocysts with modified acid-fast staining and for giardia cysts by saline–iodine preparations.

### *Enrolment of subjects*

To determine the effect of early childhood diarrhoea caused by cryptosporidium spp. and giardia spp. on social maturity, intelligence and physical growth parameters, a follow-up study was carried out at around 3 years of age between June and December 2005. Children identified as having had at least one episode of cryptosporidial or giardial diarrhoea at or below the age of 2 years and children with no documented cryptosporidial or giardial infection detected by microscopy at the time of diarrhoea at or below the age of 2 years were recruited. Informed consent was obtained from either parent at the start of the study. The study protocol was approved by the institutional review board of Christian Medical College, Vellore.

### *Assessment of social maturity, intelligence and physical growth*

Anthropometric parameters were measured monthly and recorded for all children in the birth cohort using a standardised and calibrated infantometer and weighing scales. The WHO growth reference curves were used to interpret data and calculate Z-scores. Children with height-for-age Z-scores (HAZ), weight-for-age Z-scores (WAZ) and weight-for-height Z-scores (WHZ) $<$-2 were categorised as stunted, underweight and wasted, respectively.[16]

Social maturity was assessed using the Indian adaptation of the Vineland Social Maturity Scale (VSMS).[17] The adaptive functioning is measured in the context of

eight domains including self-help general, self-help dressing, self-help eating, self-direction, occupation, locomotion, socialisation and communication. The social maturity age associated with level of functioning was calculated and then converted into a social quotient (SQ).

The Seguin Form Board Test (SFBT), a culture-fair, visuo-motor performance-based test, was administered to assess intelligence.[18] The children were asked to perform the task three times and the best time of all the three trials was used to determine a mental age which was subsequently converted to an intelligence quotient (IQ). Both the VSMS questionnaire and the SFBT were completed on the same day and administered at the study clinic in a separate room by a medical research officer trained by clinical psychologists. The research officer did not have access to the initial information about the children, including episodes of diarrhoea and socio-demographic variables.

### *Statistical analysis*

SPSS 12.0 for Windows (SPSS Inc., Chicago, IL) was used for statistical analysis. Anthropometric data were analysed using the EpiNut program in the EpiInfo 2002 software (CDC, Atlanta, GA). In order to identify significant predictors of SQ, IQ and stunting, univariate analysis was performed. A past history of any protozoan diarrhoea (either cryptosporidial or giardial), giardial diarrhoea alone and cryptosporidial diarrhoea alone were examined as predictors in the univariate analysis, in addition to other socio-demographic data collected. The data were also analysed after adjusting for socio-economic status and gender. For the social and intelligence quotient, linear regression analysis was performed for SQ and IQ, while logistic regression analysis was undertaken for stunting.

## Results

Forty of 116 children who consented to take part in the study were identified as having had cryptosporidial diarrhoea, 66 of them had giardial diarrhoea and 22 had both. Thirty-two with no documented episodes of cryptosporidial or giardial diarrhoea were also recruited. Birthweight and socio-demographic characteristics of the 116 children enrolled were comparable with the other

TABLE 1. *Comparison of socio-demographic characteristics between the study population and the birth cohort non-participants in the study.*

| Variable | Nested study $n$=116 (%) | Others $n$=336 (%) | $p$-value |
|---|---|---|---|
| Male⋆ | 64 (55.2) | 163 (48.5) | 0.22 |
| Socio-economic status⋆ | | | |
| Low | 73 (62.9) | 208 (62.0) | 0.35 |
| Middle | 40 (34.0) | 108 (32.1) | |
| High | 3 (2.6) | 20 (6.0) | |
| Maternal education⋆ | | | |
| Nil | 43 (37.1) | 96 (29.0) | 0.16 |
| 0–8 y | 57 (49.1) | 175 (52.1) | |
| >8 y | 16 (13.8) | 65 (19.4) | |
| Maternal age at birth, mean [SD]† | 24.16 [4.30] | 24.12 [4.0] | 0.92 |
| Family size, mean [SD]† | 6.33 [2.05] | 5.96 [1.94] | 0.08 |
| No. of siblings, mean [SD]† | 2.40 [1.24] | 2.19 [1.2] | 0.11 |
| Low birthweight⋆‡ | 10 (9) | 41 (12.4) | 0.53 |

⋆ $\chi^2$ test; † two-tailed $t$-test; ‡ data not available for 11 children.

children in the birth cohort (Table 1). Enrolled children with a history of protozoan (either cryptosporidial or giardial or both) diarrhoea ($n=84$) and those without ($n=32$) had comparable socio-demographic profiles (Table 2). The mean (SD) age of the children during assessment was 3.51 (0.38) years. The median (IQR) ages at the first documented cryptosporidial and giardial episodes were 1.29 (0.81–2.05) and 1.69 (1.20–2.13) years, respectively. The median (IQR) times between the last cryptosporidial and giardial episodes and time of assessment were 1.93 (1.36–2.89) and 1.64 (1.20–2.13) years, respectively.

*Social maturity*

SQ scores based on the Vineland Social Maturity Scale ranged from 13.3 to 258.0 (mean 120.1, SD 29.25). The mean (SD) SQ of children with no past history of protozoan diarrhoea was 124.97 (25.35) while that for children with a past history of protozoan diarrhoea was marginally lower at 118.22 (30.54) ($p=0.27$) (Table 3). Children with cryptosporidial and giardial diarrhoea had SQs of 118.70 (35.01) ($p=0.714$) and 116.07 (27.92) ($p=0.09$), respectively. Neither a history of giardial diarrhoea nor a history of cryptosporidial diarrhoea was a significant predictor of SQ, although the effect of giardial diarrhoea was stronger ($\beta=-8.62$, $p=0.12$) than that of cryptosporidial diarrhoea ($\beta=-1.82$, $p=0.75$) at lowering the SQ level. The results of the univariate analysis for the significant predictors of SQ are summarised in Table 3. The adjusted analysis revealed that any level of maternal education was a positive predictor of SQ score ($p=0.05$), while the presence of wasting showed a significant negative association ($p=0.03$). Being underweight as well as having a history of previous helminth infection had a strong but not significant negative effect on SQ.

*Intelligence quotient*

IQ score based on the Seguin Form Board Test was determined for 101 children. The score could not be calculated for 15 children who neither co-operated nor completed the shape-matching in SFBT. Mean (SD) IQ was 99.22 (18.79) and for those with and without protozoan diarrhoea was 97.07 (15.79) and 104.58 (24.23), respectively. Children with a past history of giardial diarrhoea (mean IQ 95.81, SD 15.71) had significantly lower IQ scores than those without a past history of giardial diarrhoea

TABLE 2. *Comparison of socio-demographic characteristics between children with and without protozoan diarrhoea.*

| Variable | History of protozoan diarrhoea $n=84$ (%) | No history of protozoan diarrhoea $n=32$ (%) | $p$-value |
|---|---|---|---|
| Male★ | 45 (53.6) | 19 (59.4) | 0.57 |
| Socio-economic status★ | | | |
| Low | 56 (66.7) | 17 (53.1) | 0.18 |
| Middle | 27 (32.1) | 13 (40.6) | |
| High | 1 (1.2) | 2 (6.3) | |
| Maternal education★ | | | |
| Nil | 32 (38.1) | 11 (34.4) | 0.30 |
| 0–8 y | 43 (51.2) | 14 (43.8) | |
| >8 y | 9 (10.7) | 7 (21.9) | |
| Maternal age at birth, mean [SD]† | 23.70 [3.89] | 25.38 [5.10] | 0.06 |
| Family size, mean [SD]† | 6.25 [2.09] | 6.53 [1.95] | 0.51 |
| No. of siblings, mean [SD]† | 2.40 [1.29] | 2.38 [1.13] | 0.91 |
| Low birthweight★‡ | 7 (8.8) | 3 (9.7) | 0.88 |

★ $\chi^2$ test; † two-tailed $t$-test; ‡ data not available for 5 children.

TABLE 3. *Predictors of social quotient in children with and without a history of cryptosporidial and giardial diarrhoea.*

| Variable | Univariate β | Univariate *p* | Adjusted⋆ β | Adjusted⋆ *p* |
|---|---|---|---|---|
| History of protozoan diarrhoea | −6.75 | 0.27 | −5.26 | 0.39 |
| History of giardial diarrhoea | −9.31 | 0.09 | −8.62 | 0.12 |
| History of cryptosporidial diarrhoea | −2.11 | 0.71 | −1.82 | 0.75 |
| No. of episodes of protozoan diarrhoea | −2.92 | 0.16 | −2.99 | 0.16 |
| History of helminth infection | −14.34 | 0.09 | −14.60 | 0.08 |
| Low birthweight | −15.17 | 0.10 | −16.05 | 0.08 |
| Maternal education | | | | |
| None | Reference | | | |
| Standards 1–8 | 11.61 | 0.04 | 11.54 | 0.05† |
| Standards 9 up to college | 26.30 | 0.002 | 24.79 | 0.01† |
| Education of head of family | | | | |
| None | Reference | | | |
| Standards 1–8 | 4.48 | 0.46 | 3.76 | 0.54 |
| Standards 9 up to college | 21.08 | 0.007 | 20.42 | 0.01† |
| Physical growth parameters | | | | |
| Stunted | −8.10 | 0.14 | −8.66 | 0.12 |
| Wasting | −20.15 | 0.04 | −20.59 | 0.03† |
| Underweight | −10.29 | 0.06 | −9.48 | 0.08 |

⋆ Adjusted for gender and socio-economic status; † $p \leqslant 0.05$.

even, when adjusted for gender and socio-economic status (Table 4). A similar effect could not be seen with cryptosporidial diarrhoea (mean IQ 100.12, SD 17.28). The results of analysis for significant predictors for the outcome IQ are summarized in Table 4. Exclusive breast-feeding positively correlated with IQ scores ($p=0.05$) while low birth weight and stunting were near significant negative predictors of IQ ($p=0.07$ and 0.09, respectively). Factors like number of family members and siblings, birth order, maternal age, stool disposal method and education of the mother or head of the

TABLE 4. *Predictors of intelligence quotient in children with and without a history of cryptosporidial and giardial diarrhoea.*

| Variable | Univariate β | Univariate *p* | Adjusted⋆ β | Adjusted⋆ *p* |
|---|---|---|---|---|
| History of protozoan diarrhoea | −7.51 | 0.07 | −6.57 | 0.11 |
| History of giardial diarrhoea | −7.84 | 0.04 | −7.58 | 0.04† |
| History of cryptosporidial diarrhoea | 1.32 | 0.74 | 1.60 | 0.69 |
| No. of episodes of protozoan diarrhoea | −2.08 | 0.15 | −2.24 | 0.12 |
| History of helminth infection | −2.42 | 0.69 | −2.22 | 0.71 |
| Low birthweight | −9.60 | 0.14 | −11.87 | 0.07 |
| Exclusive breastfeeding, mths | 2.48 | 0.04 | 2.41 | 0.05† |
| Physical growth parameters | −5.94 | 0.12 | −6.57 | 0.09 |
| Stunted | | | | |
| Wasting | −4.51 | 0.52 | −3.48 | 0.62 |
| Underweight | −2.92 | 0.44 | −2.16 | 0.57 |

⋆ Adjusted for gender and socio-economic status; † $p \leqslant 0.05$.

household did not correlate with IQ (individual data not presented).

*Physical growth parameters*

Stunting was seen in 43.1% while 8.6% of participants had wasting. In the univariate analysis, a past history of any protozoan diarrhoea, either giardial or cryptosporidial, was not a significant predictor of stunting or being underweight. However, on adjusting for socio-economic status and gender, the odds ratio (OR) (95% CI) of a past history of protozoan diarrhoea associated with stunting was 2.28 (0.92–5.61). Stunting was also strongly associated with being underweight (OR 14.84, 95% CI 5.57–39.54) or of low birthweight (OR 3.85, 95% CI 0.94–15.80). Only low birthweight was a significant predictor of being underweight (OR 3.64, 95% CI 0.88–15.10). A past history of giardial diarrhoea was also found to be significantly associated with wasting (Fisher's exact test, $p=0.04$).

## Discussion

This study analyses the effect of early childhood protozoan diarrhoea on growth, intelligence and social maturity in 3-year-old children. Previous studies have evaluated these effects at an older age. Although assessment of intelligence at a very young age is challenging, the present study is unique in evaluating the impact of protozoan diarrhoea as early as 3 years of age, and it is the first such report from India.

Cognitive function and linear growth in children are affected by environmental and health-related factors. The 1st 2 years of childhood are marked by rapid brain growth and maturation by neuronal arborisation, myelination and emergence of brain networks.[19] There is synaptogenesis and myelination in which development and pruning of thousands of synapses govern the sculpting of the brain circuitry.[20] Sensory experiences and learning alter the cortical circuitry and representations and are the basis for the neuroplasticity vividly seen in childhood.[21] Thus, the development of human intelligence depends on the hierarchical maturation of neocortical association areas as well as interactions with the environment in early life. Factors in early life such as nutrition, infection and environment affect neuroplasticity and may have long-lasting effects on the child.

In India, early childhood malnutrition including micronutrient deficiency,[22] anaemia[23] and helminthiases[24] are reported to affect cognitive development, but no previous attempts have been made to study the possible long-term impact of early childhood diarrhoea caused by protozoan pathogens. Studies in other developing countries including Brazil,[5,6,25] Peru,[4,7] Malaysia,[26] Turkey[27] and Bangladesh[28] have implicated early childhood diarrhoea caused by enteric protozoan pathogens as independent predictors of stunting. The pathogens implicated independently of one another are cryptosporidium spp.,[3,7,29] giardia spp.[26,27] and *Entamoeba histolytica*.[28] Infection by these pathogens results in adverse effects on physical fitness, cognitive function and academic achievement.[3,4,27] In addition, verbal fluency and school performance were also affected by repeated episodes of early childhood diarrhoea owing to giardia and cryptosporidium spp.[6,25]

In this study, a history of giardial diarrhoea was associated with a significantly lower IQ as early as 3 years of age, while other studies demonstrated similar effects on older school children. Giardial diarrhoea might affect growth and cognition by causing a deficiency of micronutrients such as zinc and iron,[30,31] which bind to the giardial variant surface proteins,[32] and also the malabsorption of fats and defects in the anti-oxidant system.[31] These nutritional deficiencies, along with weakness of the anti-oxidant system in the 1st years of brain development and maturation, could potentially affect neuroplasticity, in turn affecting cognitive ability.

In this study, as in others, cryptosporidial diarrhoea was not related to a decrease in cognitive function.[4] Despite the reported effect of cryptosporidiosis on linear growth faltering in children during infancy as well as a decrease in weight gain in children with both symptomatic and asymptomatic infections,[7,29] no effect on physical growth was seen in this study. Although a past history of protozoan diarrhoea was found to affect IQ as well as linear growth, there was no correlation with the number of protozoan diarrhoeal episodes (Table 4).

The predictors of intelligence and social quotients have also been evaluated and concur with other studies. Breastfeeding has a positive impact on cognition through enhanced brain growth and white matter myelination.[33] The negative correlation of poor paternal and maternal education, an absent father and low birthweight and the positive correlation of good psychosocial stimulation in infancy and cognition have been demonstrated.[34]

### *Limitations of the study*

The intelligence quotient could not be calculated for some children who did not co-operate with the Seguin Form Board test. The Vineland Social Maturity Scale was used in this study as it is the only social scale with an Indian adaptation. It is a parent-administered questionnaire and their emotional attachment to the subject might have affected the objectivity of the test. Previous studies of children in developing countries have shown that asymptomatic giardial or cryptosporidial infections were also associated with stunting and that growth faltering subsequent to giardia or cryptosporidium infection occurred even if not associated with diarrhoea.[29,35] In the present study, however, we did not examine stools of children without diarrhoea which might have affected our results and choice of controls.

Despite the limitations, this study demonstrates the negative effect of early childhood protozoan diarrhoea, especially giardial diarrhoea, on growth and cognition in Indian children as early as 3 years of age. Follow-up testing of cognitive function and growth in these children at school-age will add valuable information on the longer-term effects of protozoan diarrhoea. The role of asymptomatic giardial and cryptosporidial infections on cognitive function and growth in Indian children also needs to be assessed in future prospective studies.

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