Analysis of Different Prognostic Indicators for Malnutrition and *Shigella flexneri* Infection Among the Children in Bangladesh

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Abstract The present study investigates into some socio-economic, demographic, and nutritional features that can predispose Bangladeshi children to malnutrition and *Shigella flexneri* infection. Significant prognostic indicators associated with malnutrition were mother's illiteracy (unadjusted odds ratio, OR = 2.580; 95% confidence interval, CI = 1.134–5.867 and adjusted odds ratio, ORa, 3.814; 95% CI = 1.124–12.943), low birth weight (OR = 3.143; 95% CI = 1.2–8.232 and ORa = 2.404; 95% CI = 0.870–6.644) and poor socioeconomic status (OR = 2.549; 95% CI = 1.382–4.701 and ORa = 1.808; 95% CI = 0.852–3.838). Age was the strongest predictor for the acquisition of *S. flexneri* infection in the studied population (OR = 5.472; 95% CI = 2.583–11.592 and ORa = 5.808; 95% CI = 2.420–13.942). The severity of malnutrition was significantly ($P = 0.033$) related to seroprevalence of *S. flexneri* infection. To reduce malnutrition emphasis should be given on controlling the incidence of low birth weight, improving the literacy status especially of mothers and narrowing the gap between different socio-economic levels. Malnourished children should be examined for severity and direct intervention therapy should be given when necessary.

Keywords Children · Malnutrition · *Shigella flexneri* · Bangladesh

Introduction

Worldwide about 852 million people have been reported to be undernourished. In Bangladesh the situation is particularly worse. Nearly one-half of all children are either underweight or stunted [1, 2]. Low birth weight [3] is especially important among various factors that contribute to the poor health status in Bangladeshi children. Although breast feeding is common in Bangladesh most mothers still do not feed their breast exclusively for 4 months, often beginning supplemental feeding at an early age, making their children more susceptible to infection and malnutrition [4]. Maternal education also plays a very important role in good child care practices and can be considered as women's resources for nurturing their children. Moreover, educational attainment of women is strongly related to fertility levels. In Bangladesh, the current situation is such that women with no formal education would give birth to an average of 4.1 children in their lifetime, compared with 2.4 for women with at least some secondary education, a difference of 42% [5]. There are also inequities between socio-economic levels. In accordance with the Bangladesh Household Income Expenditure Survey data of 2000, about 45% of the population lives below poverty line [6].

Malnutrition is synergistically related to shigellosis [7, 8]. Shigellosis causes a protein-losing enteropathy that can exacerbate malnutrition and lead to death [9]. At present the predominant species in Bangladesh is *S. flexneri* [10]. Because of low infective dose minor hygienic errors allow fecal-oral spread [11]. Person-to-person transmission is common among toddlers, who are mobile but have not yet

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developed hygienic practices adequate to prevent transmission [12]. Currently, no vaccine against *Shigella* infection exists [13]. Only option is the use of antibiotics which often contributes to the increasing emergence of resistance phenotypes [7].

To counter malnutrition as well as *Shigella* infection in a population, it is important to know their incidence, trends, and differentials. This work aims to investigate the risks posed by the incidence of low birth weight, mother's literacy status, family's socio-economic background, breastfeeding practice in the first 4 months of life and gender on acquiring *S. flexneri* infection and malnutrition among the children in Bangladesh.

Materials and Methods

A case control study was conducted from September 2006 to May 2007 at a mother and children based hospital (Ad-Din Hospital) in Dhaka, Bangladesh. Children, in the age group of 0–12 years, attending for outdoor treatment (common infectious and noninfectious illnesses) or admitted in the nutrition ward of the same hospital and whose weight-for-age were <90% of median of the NCHS, weight-for-height $\leq$ 90% of median of the NCHS, height-for-age <95% of the median of the NCHS, and mid arm circumference <14 cm [5, 14, 15] were enrolled in this research as malnourished cases. The case population was further graded into mild, moderate and severe based on height-for-age (%), weight-for-age (%) and weight-for-height (%) [5, 15, 16]. The age matched control population comprised of the healthy and nonmalnourished children (0–12 years) who attended the same hospital for routine vaccination or blood group testing. Their weight-for-age were >90% of median of the NCHS, weight-for-height were $\geq$ 90% of median of the NCHS, height-for-age >95% of median of the NCHS and mid arm circumference were >14 cm.

Verbal, informed consent of the child's parents was obtained in both cases and controls. Information on socio-demographic and nutritional factors was collected by questioning the person attending with the children through a structured questionnaire. Each participant was provided with an identification (ID) number which was subsequently used for the serodiagnosis, data entry and analysis. Children up to 12 months were included as infants and aged more than 12 months were included as older age group. Birth weight of 2.5–4 kg was taken as normal and less than 2.5 kg as low birth weight [15]. History of breastfeeding was also recorded. A mother was considered to be educated if she received any formal schooling. Socioeconomic level was assessed by using the international poverty line (monetary cut-off points separating the poor from the non-

poor) proposed by the World Bank set at US\$1.08 per day per capita in purchasing power parity (PPP) terms [17].

Sera Preparation

Three millilitre of blood was taken into sterile glass tube. The tubes were kept at a slanted position and allowed to clot for 1 h. Serum was transferred to sterile eppendorf tube with a Pasteur pipette. The tubes were centrifuged (Eppendorf, Germany; Model: 22331) at 3,000 rpm for 10 min to settle the remaining erythrocytes down and clear serum was collected into sterile eppendorf. The serum was stored at -20°C until use [18].

Whole Cell Antigen Preparation

Shigella flexneri ATCC 12024 (serotype 4b) cells were grown in nutrient agar plates and harvested (transferred to a sterile tube using a sterile loop). Bacterial cells were suspended in phosphate buffered saline (PBS) containing 1% (v/v) formalin and kept at 4°C for 1 h. Cells were washed four-times in PBS to remove the formalin and finally, a suspension of 1 mg cells ml^{-1} PBS was made [19].

In-house ELISA Assay

This study employed in-house ELISA to detect antibody against *S. flexneri* in sera of the studied children. Briefly, 100 μl of formalin-fixed bacterial antigen (diluted to 1 μg per 100 μl of coating buffer) was added to each well. The plates were covered with plate sealer and incubated overnight at 4°C . Next day, the plates were washed three times with PBS containing Tween-20 (0.05%). Blocking was performed with 200 μl of 1% (w/v) bovine serum albumin (BSA) in PBS followed by incubation at 37°C for 30 min. The PBS-BSA was discarded. Washing was continued for 3 times with PBS–Tween-20. Then, 100 μl of diluted serum sample (1:500 in PBS) was added to all the wells except A1 and B1. These wells (A1 and B1) were marked as blank. The plate was covered and incubated at room temperature for 2 h. The plate was washed three times as described above and 100 μl of diluted (1:30,000 in PBS) antibody conjugate was added [Rabbit antihuman polyvalent immunoglobulin (whole molecule)—Peroxidase containing IgG fraction only (SIGMA)], and incubated at room temperature for 2 h. The plate was washed three times with PBS–Tween-20 and 200 μl of enzyme substrate (H_2O_2 –OPD in 0.1 M sodium citrate buffer) was added to each well. The plate was kept away from light and optical density (OD_{490}) was estimated by an ELISA reader (BIORAD, Japan, model no.680) exactly after 30 min.

Cut-off value for the HRP (Horseradish peroxidase) substrate system was calculated by comparing values of 28

positive and eleven negative controls with values of the same controls recorded by previous scientists [20] in our research group using AP [Alkaline Phosphatase (SIGMA)] substrate system. Accordingly, absorbance values of ≥ 0.1 were considered as indicative of significant levels of anti-*S. flexneri* antibodies or cut-off value for HRP system. For the AP system [20], the secondary antibody was Goat anti-human polyvalent antibody conjugated with alkaline phosphatase (SIGMA) diluted 1:800 times in PBS and the enzyme substrate was p-nitrophenyl phosphate in diethanolamine buffer whose reaction colour was measured at 405 nm.

Each test serum was analyzed in duplicate wells. For each ELISA plate, the average of blank values was subtracted from OD value for each test serum.

Statistical Analysis

To examine statistical relationships between classifications, joint frequency distribution of sample respondents in accordance with nutritional status or *S. flexneri* infection and other socio-demographic factors was made. Significance test was then performed using χ^2 statistic. Fisher's exact test was used in case of sparse data. Both univariate and multivariate logistic regression models were fitted; the results in the univariate model describe the gross effect, while the results in the multivariate model describe the net effect after controlling for the effects of other variables in the model. A variable was included in the logistic model when its *P* value was below 0.4, with the objective of excluding any variable that could have plausible effect in modifying the result. In the model the variable that emerged with a *P* value of <0.05 was taken as significant and one with a *P* value <0.001 was considered to be highly significant. When a measure was missing for a child, the child was excluded only in the analysis for that measure but was included in all other analysis.

Result and Discussion

In this study 89 malnourished cases were compared with 99 healthy, well nourished children. The majority of the children population was older than 1 year. Among the mildly malnourished children 9 were infants and 44 above 1 year. There were 8 infants and 17 children aged above 1 year in moderately malnourished category and 5 infants and 6 children aged above 1 year in the severely malnourished group.

Risk Factors for Acquisition of Malnutrition

The category of children according to their nutritional status (with level: case children and control children) and different socio-demographic characteristics have been

arrayed in Table 1 in order to test the null hypothesis (H_0 : there is no association between malnutrition and various socio-demographic factors). The *p* values indicated that low birth weight, mother's illiteracy and poor socioeconomic status were significantly related to malnutrition ($P < 0.05$).

After fitting the variables in the logistic model (Table 2), mother's education showed a significant impact ($P < 0.05$). Illiterate mothers carried a 2.6-fold risk in generating poor nutrition in their children. But in the presence of interaction with other variables, mother's illiteracy emerged to have about 4-fold increased risk ($P = 0.032$) for developing malnutrition in the studied population. Significant impact of mother's illiteracy on malnutrition demonstrated in the current investigation coincides with an earlier study conducted by Ali et al. [21]. Poverty acts to limit access to care, amount of foods and other amenities needed for proper growth of children [22]. The present data reveals that children of poor background suffer from higher rates of malnutrition than the non-poor children do (unadjusted odds ratio, OR = 2.55; 95% CI = 1.382–4.701, *P* value, 0.003; adjusted odds ratio, ORa = 1.801; 95% CI = 0.852–3.838). Previously Giasuddin et al. [6] observed similar relationship between socioeconomic condition and nutritional status of children. Low birth weight (LBW) remains a significant contributor to infant mortality in many developing countries. In Bangladesh, half of all babies are born with weight of <2.5 kg [4]. Poor nutrition both before and during pregnancy is recognized as an important cause of LBW. Even if a LBW baby survives infantile stage there is obvious likelihood of growth retardation and illness throughout childhood, adolescence and into adulthood [2, 22]. In the examined population, LBW was significantly (<0.05) associated with three-times higher risk of acquiring malnutrition before adjustment and 2.4-fold higher risk after adjustment while *P* value shifted from 0.020 to 0.091. The vulnerability of female sex to be malnourished had borderline significance (OR = 1.651; *P* = 0.089) independently of other variables but the magnitude of significance decreased after controlling for other variables. Presence of anti-*S. flexneri* antibody was associated with elevated risk of acquiring malnutrition (OR = 1.38; ORa = 1.2) both before and after adjustment for other variables. Lack of exclusive breast feeding practice was also related to increased risk of acquiring malnutrition (OR = 1.607; ORa = 1.01) in the current investigation. Mother's knowledge and practice regarding the duration of exclusive breastfeeding are poor in Bangladesh and pre-lacteal feeds like honey or sweetened water etc. are common in this region. Giving colostrum as the baby's first food is still ignored. In a study reported by Ahmed et al. [23] only 15% of mothers were observed to breastfeed exclusively their 0–5 months-aged infants.

Table 1 Comparison of socio-demographic and nutritional variables among malnourished (case) and non-malnourished (control) children (0–12 years) in Bangladesh

Variables	Number (%) of the children		<i>P</i> value (χ^2)
	Case children (89)	Control children (99)	
Presence of anti- <i>S. flexneri</i> antibody			
No	16 (9)	23 (12)	0.375
Yes	73 (39)	76 (40)	
Birth weight			
≥2.5 kg	48 (35)	66 (48)	0.016
<2.5 kg	16 (12)	7 (5)	
Exclusive breast feeding			
Yes	61 (32)	77 (41)	0.152
No	28 (15)	22 (12)	
Sex of the child			
Male	42 (22)	59 (31)	0.089
Female	47 (25)	40 (21)	
Mother's education			
Literate	69 (37)	89 (47)	0.021
Illiterate	20 (11)	10 (5)	
Socioeconomic status			
Non-poor ^a	24 (13)	48 (26)	0.002
Poor ^b	65 (35)	51 (27)	

Values in bold are significant at 5% level

[1 US\$ = 69 Taka (Bangladesh currency)]

^a per day per capita income above 1.08\$

^b per day per capita income below 1.08\$

Risk Factors for Acquisition of *S. flexneri* Infection

Investigation into the study variables for differences in their association with *S. flexneri* infection using χ^2 test revealed that infancy had highly significant relationship with *S. flexneri* infection ($P < 0.001$) (Table 3). Seroprevalence of anti-*S. flexneri* antibody was higher in the children group older than 1 year (88%) as compared to infants (57%). The logistic regression model indicated that children aged >1 year had about six-times higher risk to acquire *S. flexneri* infection (OR = 5.472*** 95% CI = 2.583–11.592 and OR_a = 5.808***; 95% CI = 2.420–13.942). Consistent to this is the result of stratification of the children into smaller groups by age in both malnourished and nonmalnourished children (Fig. 1). Seroprevalence of anti-*S. flexneri* antibody in well nourished children (control) increased with increasing age with a little decline in the 4–6 years of age-group. But there was peak of the seroprevalence in the 3–12 months age-period and again at 6–8 years age-period in the malnourished children. The most prominent difference of the seroprevalence of anti-*S. flexneri* antibody between the malnourished and

Table 2 Univariate and multivariate risk-factor analysis for malnourishment

Explanatory variables	Unadjusted odds ratio (OR) (95% confidence interval)	Adjusted odds ratio (OR) ^a (95% confidence interval)
Presence of anti- <i>S. flexneri</i> antibody		
No	1	1
Yes	1.381 (0.676–2.821)	1.211 (0.507–2.891)
Birth weight		
≥2.5 kg	1	1
<2.5 kg	3.143** (1.2–8.232)	2.404* (0.870–6.644)
Exclusive breast feeding		
Yes	1	1
No	1.607 (0.837–3.083)	1.009 (0.426–2.387)
Sex of the child		
Male	1	1
Female	1.651* (0.926–2.943)	1.298 (0.611–2.759)
Mother's education		
Literate	1	1
Illiterate	2.580** (1.134–5.867)	3.814** (1.124–12.943)
Socioeconomic status		
Non-poor	1	1
Poor	2.549** (1.382–4.701)	1.801 (0.852–3.838)

^a Adjusted for all other variables in the model

* $P < 0.10$. ** $P < 0.05$. *** $P < 0.001$

nonmalnourished children was observed in the 3–6 months age-group of the children population.

In a study reported by Ahmed et al. [24] substantially increased risk of shigellosis after infancy with peak in the second year of life was observed. The higher seroprevalence in children aged more than 1 year may be due to the fact that children at this age are more exposed to contaminated food, kitchen utensils, and bottles. Touching food with contaminated hands has been the reason of many outbreaks of foodborne diseases. For those pathogens that have a low minimum infective dose and for which the human body is the main reservoir as *Shigella* spp., contaminated hands are a particularly important risk factor [25]. An elevated proportion of mother or the food preparer in Bangladesh is unaware about safe food handling. For example, they often keep food open at ambient temperatures for a long time after preparation. Again insufficient water is an important reason for not washing hands or utensils properly. Toddlers and children at preschool age are difficult to train the hygienic practice. Furthermore, school going children keep their tiffin foods in school bag for some hours before consumption which may promote *Shigella* growth specially in hot climate countries like Bangladesh. The importance of proper food handling practice in addition to safe water in the risk analysis for

Table 3 Seroprevalence of anti-*Shigella flexneri* antibody by socio-demographic and nutritional variables in under 12 children in Bangladesh ($n = 188$)

Variables	Number of children	Children with anti- <i>S. flexneri</i> antibody (seroprevalence of anti- <i>S. flexneri</i> antibody) (%)	<i>P</i> value (<i>X</i> ²)
Severity of malnutrition			
Normal	99	76 (77)	0.033
Mild	53	48 (91)	
Moderate	25	19 (76)	
Severe	11	6 (55)	
Birth weight			
≥2.5 kg	114	88 (77)	0.735
<2.5 kg	23	17 (74)	
Exclusive breast feeding			
Yes	138	114 (83)	0.060
No	50	35 (70)	
Sex of the child			
Male	101	81 (80)	0.731
Female	87	68 (78)	
Mother's education			
Literate	158	128 (81)	0.173
Illiterate	30	21 (70)	
Socioeconomic status			
Non-poor	72	59 (82)	0.474
Poor	116	90 (78)	
Infancy			
Infants	54	31 (57)	0.000
Non-infants	134	118 (88)	

Values in bold are significant at 5% level

malnutrition and diarrhea has been reviewed by Motarjemi et al. [25].

The present study reports seroprevalence of anti-*S. flexneri* antibody at about 79.3 percent level among Bangladeshi children ($n = 188$). Significant association was found (Table 3) between severity of malnutrition and

seroprevalence of anti-*S. flexneri* antibody ($P = 0.033$) in our work. Mildly malnourished subset exhibited the highest seroprevalence (91%) of anti-*S. flexneri* antibody (OR = 2.905**; 95% CI = 1.035–8.158 and ORa = 2.255; 95% CI = 0.768–6.624) ($P = 0.043$) whereas 77% of nonmalnourished group contained anti-*S. flexneri* antibody. Diarrheal disease has been previously related to poor nutritional status [8, 26]. However, the moderate group of malnourished children in this research showed seroprevalence (76%) similar to the nonmalnourished normal children (OR = 0.958; 95% CI = 0.342–2.683 and ORa = 1.132; 95% CI = 0.365–3.516). The severely malnourished children contained the lowest seroprevalence of anti-*S. flexneri* antibody (55%) which contrasts to some other reports that shigellosis burden increases with severity of malnutrition [27]. Antibody response to *Shigella* spp. is developed solely against LPS on the cell surface [28]. Malnutrition greatly hampers immune competence with concomitant reduced antibody production particularly to polysaccharide antigens [29]; this could explain the decreasing seroprevalence with severity of nutritional deterioration reported in our work.

Exclusive breast feeding in the first 4 months of life had almost significant association with *S. flexneri* infection ($P = 0.060$). Low birth weight, sex of the child, mother's education and socioeconomic status did not reveal significant association to seroprevalence of *S. flexneri* infection in this study population. A substantial protective effect of breast feeding on *Shigella* diarrhoea has been documented previously [30]. In the current research, association between exclusive breast feeding and *S. flexneri* infection reached significance at 10% level. Although, the absence of breast feeding practice exclusively in the first 4 months of life failed to show risk to acquire *S. flexneri* infection without adjustment, the adjusted odds ratio (ORa = 1.319; 95% CI = 0.507–3.430) pointed to this practice being worth to be valued.

Necessary actions that could be implicated from the present study to reduce malnutrition and shigellosis are:

Fig. 1 Seroprevalence of anti-*S. flexneri* antibody in different age strata of malnourished (case) and nonmalnourished (control) children in Bangladesh

special attention to pregnant women for lowering the low birth weight problem, improved maternal education and poverty alleviation policies. Direct nutrition interventions are needed to assist those already affected by malnutrition.

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