

## Association of Secretor Status with Enteropathy and Growth among Children in Bangladesh Aged 1–24 Months

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**Abstract.** Secretor status refers to the ability of an individual to secrete blood group antigens into body fluids and onto the different epithelial surfaces. Concurrent findings have demonstrated an association of the secretor status of children with susceptibility to a plethora of enteropathogens. We aimed to determine a possible association of secretor status of children with childhood enteropathy, an important causal factor for childhood growth failure. Participants of the Malnutrition and Enteric Disease (MAL-ED) birth cohort study from the Bangladesh site were enrolled along with their mothers. Saliva was analyzed for determining blood groups and secretor status of the children and their mothers by using an in-house ELISA. Approximately 59% of children and 65% of mothers were found to be secretor positive. Secretor-positive children were found to have a significantly positive association with alpha-1-antitrypsin ( $\beta$ -coefficient: 0.11, 95% CI: 0.07, 0.21,  $P < 0.01$ ) and with environmental enteric dysfunction score ( $\beta$ -coefficient: 0.32, 95% CI: 0.29, 0.65,  $P = 0.05$ ). However, despite a negative effect size, secretor-positive children did not show any statistical significance with length-for-age and weight-for-age z scores (LAZ and WAZ), respectively. Our findings indicate toward the genetic factor of secretor status of children being associated with childhood growth faltering, through increased susceptibility to distinct enteropathogens and the consequent development of enteric inflammation and enteropathy among children. However, these findings are only applicable in Bangladeshi settings and thus need to be validated in several other similar settings, to establish a possible relationship between the secretor status of children with enteropathy and resulting childhood growth failure.

### INTRODUCTION

Environmental enteric dysfunction (EED) refers to the histological alterations of the architecture of the intestine that is usually specified by the increase in the depth of crypts, infiltration of lymphocytes, and decreased surface area of the intestinal mucosa.<sup>1</sup> Continual immune activation as a result of chronic exposure to diarrheal enteropathogens, and enteric inflammation raised permeability of the intestine are the distinctive indications of EED.<sup>2,3</sup> However, a phenomenon known as dysbiosis that indicates toward immaturity of the gut microbiome and depletion of age-specific beneficial members of the gut microbiota such as *Bifidobacterium infantis* during the first 6 months of life and *Prevotella copri* during the postweaning period have been reported to contribute to enteric inflammation and growth retardation, independent of pathogen carriage.<sup>4</sup> Nevertheless, early childhood growth failure has been attributed to the chronic exposure to diarrheal enteropathogens and consequent development of the features of EED.<sup>1</sup> Concurrent reports from studies carried out in the settings of the MAL-ED study have shown that asymptomatic infection by diarrheal enteropathogens such as *Campylobacter jejuni/coli*, EAEC, enterotoxigenic *Bacteroides fragilis* contribute to enteric inflammation.<sup>5–7</sup>

Upper gastrointestinal (GI) endoscopy is widely regarded as the gold standard for the diagnosis of EED.<sup>8</sup> Although a few studies have used upper GI endoscopy to study the pathophysiology of EED,<sup>9,10</sup> the invasive nature of the procedure and the requirement of specialized clinical settings and expertise renders it infeasible to be carried out among

children in resource-limited settings.<sup>8</sup> Concurrent findings show that several fecal biomarkers, namely myeloperoxidase (MPO; indicates intestinal inflammation), neopterin (NEO; indicates intestinal inflammation), and alpha-1 antitrypsin (AAT; indicates loss of enteric proteins and intestinal permeability), are associated with EED and thus have been proposed as noninvasive alternatives for the diagnosis of EED.<sup>11–13</sup>

Secretor status refers to the ability or inability of an individual to secrete histo-blood group antigens into body fluids (such as saliva and breast milk) and/or onto the different epithelial surfaces. The fucosyltransferase-2 (FUT-2) gene that encodes the FUT-2 or the secretor enzyme allows for secretion of the histo-blood group antigens into the body fluids and/or onto the various epithelial surfaces.<sup>14–16</sup> The histo-blood group antigens (ABO) are structurally defined as the immunodominant glycans and are synthesized by the glycosyltransferases, which, in turn, are encoded by the ABO gene.<sup>17</sup> On the other hand, individuals who do not express the ABO antigens in body fluids and/or epithelial surfaces are called “nonsecretors”.<sup>18,19</sup> The Lewis blood group system is another blood group system comprising the antigens Lewis a and b forming the groups: Lewis A, Lewis B, Lewis A+B+, and Lewis Negative.<sup>20</sup> The FUT-3 gene encodes the FUT-3 or Lewis enzyme, which is essential for Lewis antigen formation. The activity of the FUT2 enzyme is also required for the production of the Lewis b (Le b) antigen.<sup>18</sup> Homozygous inactivating mutations in the FUT2 gene result in the nonsecretor phenotype and so nonsecretors are usually Lewis A and secretors tend to be Lewis B. Individuals with inactive FUT3 enzyme do not express Lewis a and b, and are termed Lewis-negative.<sup>21</sup>

Concurrent findings have shown possible associations between the positive secretor status and increased susceptibility to a number of enteropathogens, including norovirus genotype GI<sup>22</sup> as well as to rotavirus,<sup>23</sup> *Helicobacter pylori*,<sup>24,25</sup> and *C. jejuni/coli*.<sup>26</sup> Individuals who are secretors

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present their blood group antigens onto the epithelial surface of the intestinal mucosa, which serve as attachment sites for these enteropathogens.<sup>27–29</sup> However, potential links between the secretor status and enteropathy in children have not been investigated till date. In this study, we have attempted to establish a possible association between the secretor status and enteropathy among young children in Bangladesh, to elucidate genetic cues for childhood enteric inflammation. For our study, we have used the settings of the MAL-ED birth cohort study and have enrolled the children who had been previously enrolled in the MAL-ED study at the Bangladesh site. Additionally, for the purpose of deciphering a possible intergenerational cue for childhood enteric inflammation and among the study participants we also enrolled the mothers of the study participants and also assessed the secretor status of the mothers.

## MATERIALS AND METHODS

### Study site, study participants, and ethics statement.

This present study involved the participants who were enrolled at the Bangladesh site of the MAL-ED birth cohort study. The detailed study protocol for the Bangladesh site of the MAL-ED study, conducted in the Bauniabadh slum area in Mirpur, Dhaka, has been described elsewhere.<sup>30</sup> Briefly, a total of 265 participants of either sex were enrolled in the study between November 2009 and February 2012. The study participants were enrolled within 17 days of birth and followed up to 24 months of life. Among the 265 participants enrolled in the study, 210 completed the 24-month follow-up. For our current study, conducted between August and October of 2017, we reenrolled the mothers and their children who had participated in the MAL-ED birth cohort study at the Bangladesh site. Only 184 children and 152 mothers were available for our current study, because some of the children and mothers originally enrolled in the MAL-ED Bangladesh site had already migrated to distant places. The number of migrants was equally distributed across all zones of the study area. Moreover, the similar number of male and female participants had migrated during the reenrollment. Henceforth, there was minimal risk of selection bias during reenrollment. Our study was approved by the Institutional Review Board (IRB) of International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) on August 17, 2017. The IRB of icddr,b comprises the Research Review Committee (RRC) and the Ethical Review Committee (ERC). Written informed consent was obtained from mothers or legal guardians of the children before the enrolment of the children and their mothers in our study.

**Collection of anthropometric, sociodemographic, and morbidity data.** Anthropometric measurements were made every month from enrolment till 24 months of age, using standard scales (Seca GmbH & Co. KG., Hamburg, Germany). Sociodemographic data on the child's birth, including birth weight, anthropometric indices at birth, presence of siblings, and other maternal characteristics were collected at enrollment.<sup>31</sup> A detailed account of any morbidity and child feeding practices were obtained during household visits, done twice weekly.<sup>32</sup>

Socioeconomic data were collected at every 6 months, starting from when the participants were 6 months old. Consequently, Water, sanitation, hygiene, Asset, Maternal

education, and Income index (WAMI score), ranging from 0 to 1, an index of socioeconomic status of the households,<sup>33</sup> was calculated; whereby a superior socioeconomic status was indicated by a higher WAMI score.<sup>34</sup> According to the WHO guidelines, an improved sanitation facility was described as one that hygienically separated human excreta from human contact, and an improved drinking water source was defined as one that by the nature of its construction adequately protected the source from outside contamination, in particular from fecal matter.<sup>35</sup> Treatment of drinking water was defined as filtering, boiling or addition of bleach.<sup>36</sup>

**Collection of biological specimens.** For our current study, saliva samples were collected from mother and child pairs using Oracol saliva collecting swabs (Malvern Medical Developments, Worcester, UK) (sponge-tipped samplers) after having their mouths rinsed 3–4 times with drinking water thoroughly for about 2 minutes for proper cleaning. The samplers were handled like toothbrushes and rubbed against gums and tongue for about a minute or until soaked in saliva. The samplers were then reinserted into the Oracol tubes and quickly transported from the field site to the laboratory at icddr,b, maintaining a cold chain ensured by the use of cooler boxes with ice packs and a digital thermometer to ensure the maintenance of a steady temperature of 2–8°C inside the cooler boxes.

Previously, from November 2009 to February 2012, our community research staff collected nondiarrheal stool samples on a monthly basis from enrolment of the participant after birth until 12 months of age. From 12 months of age, stool samples were collected once after every 3 months until 24 months of age. During collection of the stool samples, no fixative was added to the stool samples, and the raw, unprocessed stool aliquots were stored in –80°C freezers before further laboratory testing.

**Determination of secretor status.** Secretor status of the children and their mothers were determined by an in-house ELISA technique, following laboratory guidelines described elsewhere.<sup>37,38</sup> In brief, the saliva samples were diluted using 1X phosphate-buffered saline (PBS), incubated in water bath for 10 minutes and introduced to the wells of an empty binding ELISA plate; following which the ELISA plates were incubated overnight in a humidity chamber at 4°C. In the next day, ELISA was carried out using anti-Le a, anti-Le b, and anti-A, B, O antibodies. The ELISA plates were read at 450 nm using a plate reader and a cutoff value of 0.10 was used for the Lewis and histo-blood group antigens, as described in previous literature.<sup>37,38</sup> Any value equal to or above 0.10 was designated as positive. From this data, individual blood groups and secretor statuses were determined. Individuals who were positive for only Lewis A were classified as “nonsecretors” while those testing positive for Lewis B antigens along with Lewis A or the histo-blood group antigens A, B, or O were classified as “secretors.” The secretor status of some individuals (28 children and 16 mothers) could not be determined by this method, due to the optical density values of both Lewis A and Lewis B were below the cutoff value of 0.10 and thus the secretor status of these individuals was classified as “inconclusive.”

**Determination of biomarkers of enteric inflammation and detection of enteropathogens.** Enteric inflammation was assessed by measuring the levels of MPO (Alpco, Salem, NH), NEO (GenWay Biotech, San Diego, CA), and

alpha-1-anti-trypsin (Biovendor, Chandler, NC) from the monthly nondiarrheal stool samples by using quantitative commercial ELISA kits. Environmental enteric dysfunction score, ranging from 0 to 10, was calculated from the three biomarkers, as described previously,<sup>39</sup> and was used as the noninvasive indicator for childhood enteropathy. Categories were assigned with the values 0 (low), 1 (medium), or 2 (high). Myeloperoxidase, NEO, and AAT values were log-transformed before subsequent analysis. The formula used for the calculation of the EED score is as follows<sup>13</sup>:

$$\text{EED score} = 2 \times \text{AAT category} + 2 \times \text{MPO category} + 1 \times \text{NEO category}$$

On the other hand, a customized multiplex real-time polymerase chain reaction (PCR) platform involving a compartmentalized primer-probe assay system, known as TaqMan Array Cards (TAC), was used for the detection of enteropathogens.<sup>40,41</sup> A cycle threshold (Ct) value of 35 was considered as the cutoff value, whereby values lower than 35 were considered to be positive for the particular enteropathogen.

**Statistical analyses.** All statistical analyses were done using STATA 13 (StataCorp LLC, College Station, TX). Descriptive characteristics were represented by either mean with SD or by frequency and percentage. Student's *t* test or  $\chi^2$  test was done to compare the general characteristics between the secretor and nonsecretor children, depending on the nature of the data. Multivariate generalizing estimating equation (GEE) model was used to assess the potential association between secretor status of the study participants and the enteric inflammation as indicated by the inflammatory biomarkers: MPO, NEO, and AAT as well as the EED scores. The multivariate GEE was after adjusting for relevant covariates with a *P* value  $\leq 0.2$  in bivariate analysis. These covariates included sex of the child, age of the child, birth weight, duration of exclusive breastfeeding, maternal age, weight-for-age z score (WAZ) at enrolment, WAMI, and detection of enteropathogens associated with the secretor status of children. Previous findings have shown that the detection of several enteropathogens, namely norovirus genogroup II, rotavirus, typical EPEC, ETEC, *C. jejuni/coli*, *Cryptosporidium* sp., *Giardia* sp., *Vibrio cholerae*, and *H. pylori*, was associated with the secretor status of children<sup>37</sup> and so the detection of these enteropathogens was also adjusted in the final multivariate GEE model done to assess the association between secretor status of children with the enteric inflammation.

We also used a multivariate GEE model to evaluate the association between the secretor status of children with the anthropometric indices of: length-for-age z score (LAZ) and WAZ. The covariates that were adjusted in this multivariate GEE model included sociodemographic characteristics such

as sex of the child, age of the child, birth weight, WAMI score, maternal height, maternal weight, and LAZ at enrolment. Previous studies conducted in the similar settings have shown that these sociodemographic characteristics are associated with childhood growth.<sup>7,42-44</sup> Enteropathogens associated with childhood malnutrition, namely EAEC, *Campylobacter* sp., ST-ETEC, *Shigella* sp., Norovirus genogroup I, *Giardia* sp.,<sup>45</sup> were also included in this multivariate GEE model constructed to evaluate association between secretor status and LAZ and WAZ of children. In all the final multivariate GEE models, associations were considered to be statistically significant only when the *P* value was less than 0.05.

## RESULTS

**Distribution of secretor status among children and mothers.** Table 1 shows the distribution of the secretor status of the children enrolled in the MAL-ED study and their mothers. One hundred and eighty-four children who were originally enrolled in the MAL-ED study at the Bangladesh site were available for our study. Among them, 108 children were found to be secretors and 48 children were found to be nonsecretors. On the other hand, we were able to collect saliva samples for 152 mothers, among whom 101 mothers were found to be secretors and 35 mothers were found to be nonsecretors. We were unable to determine the secretor status for 28 children and 16 mothers using the in-house developed ELISA-based approach and so the secretor status of these children was deemed to be inconclusive.

**Sociodemographic characteristics of the study participants based on their secretor status.** We also analyzed the sociodemographic characteristics of the study participants based on their secretor status (Table 2). The percentage of male children was higher among the nonsecretors compared with the secretors (56.5% versus 50%). Additionally, the mothers of the secretor children were significantly less uneducated compared with the mothers of the nonsecretor children (*P* = 0.04). The monthly family income was also significantly lower among the secretor children (*P* < 0.01). Moreover, the percentage of secretor mothers among the secretor children (65.7%) was significantly higher (*P* < 0.01) than the percentage of secretor mothers among nonsecretor children (41.7%).

**Distribution of the enteric inflammatory biomarkers between the secretors and nonsecretors from 1 to 24 months of age.** Figure 1 shows the distribution of the levels of the enteric inflammatory biomarkers, namely MPO, NEO, and AAT among the secretors and nonsecretors from 1 to 24 months of age. In general, the levels of MPO and NEO were greater than that of AAT at all time intervals among both the secretors and nonsecretors. When compared between the

TABLE 1  
Distribution of secretor status among mother and children enrolled at the MAL-ED Bangladesh site

	Secretor status					
	Secretor		Nonsecretor		Inconclusive	
Children (n, %)	108 (58.7%)		48 (26.1%)		28 (15.2%)	
	Male	Female	Male	Female	Male	Female
	54 (50%)	54 (50%)	26 (54.2%)	22 (45.8%)	15 (53.6%)	13 (46.4%)
Mother (n, %)	101 (64.7%)		35 (22.4%)		16 (10.3%)	

TABLE 2  
Comparison of general characteristics of the study participants enrolled at the MAL-ED Bangladesh site on the basis of their secretor status

Sociodemographic characteristics	Secretor (N = 108)	Nonsecretor (N = 48)	P value
Male sex*	54 (50%)	26 (54.2%)	0.428
Days of exclusive breastfeeding†	146.3 ± 41.6	146.9 ± 36.2	0.464
Birth weight (kg)†	2.77 ± 0.43	2.82 ± 0.40	0.295
Weight-for-age z score at enrollment†	-1.36 ± 0.97	-1.32 ± 0.89	0.408
Length-for-age z score at enrollment†	-1.2 ± 1.11	-0.98 ± 0.86	0.260
Length-for-age z score at 24 months†	-2.20 ± 0.95	-2.06 ± 0.89	0.205
Weight-for-age z score at 24 months†	-1.82 ± 0.89	-1.67 ± 0.96	0.176
Maternal age (years)†	25 ± 5.06	24.5 ± 4.95	0.725
Maternal weight (kg)†	48.1 ± 6.96	50 ± 8.92	0.904
Maternal height (cm)†	148.9 ± 5.19	149.3 ± 4.51	0.368
Maternal educational level < 6 years*	69 (63.9%)	24 (50%)	0.04
Mother has less than three living children*	81 (75%)	39 (81.3%)	0.285
Routine treatment of drinking water*	68 (63%)	29 (60.4%)	0.839
Monthly income < \$150*	25 (23.2%)	22 (45.8%)	< 0.01
Secretor mother*	71 (65.7%)	20 (41.7%)	< 0.01

\* Data represented as n, %.  
† Data represented as mean ± SD.

secretors and nonsecretors, the levels of MPO and NEO remained comparable between the secretors and the nonsecretors. However, the levels of AAT were found to be higher among the secretors at each of the time intervals, except for month 10.

**Association of secretor status of children with childhood enteropathy and childhood growth.** Table 3 indicates the results of the multivariate GEE analysis showing association of the secretor status of the study participants with childhood enteropathy, as denoted by the enteric inflammatory

biomarkers: MPO, NEO, and AAT, as well as the EED score. After adjusting for relevant covariates including sex of the child, age of the child, birth weight, duration of exclusive breastfeeding, maternal age, WAZ at enrolment, WAMI, and detection of enteropathogens associated with the secretor status of children, we found a significant positive association between AAT levels and the EED score with the secretor-positive status of the children. Our findings indicate that among the secretor children the AAT levels and the EED scores are likely to be greater than that among nonsecretor

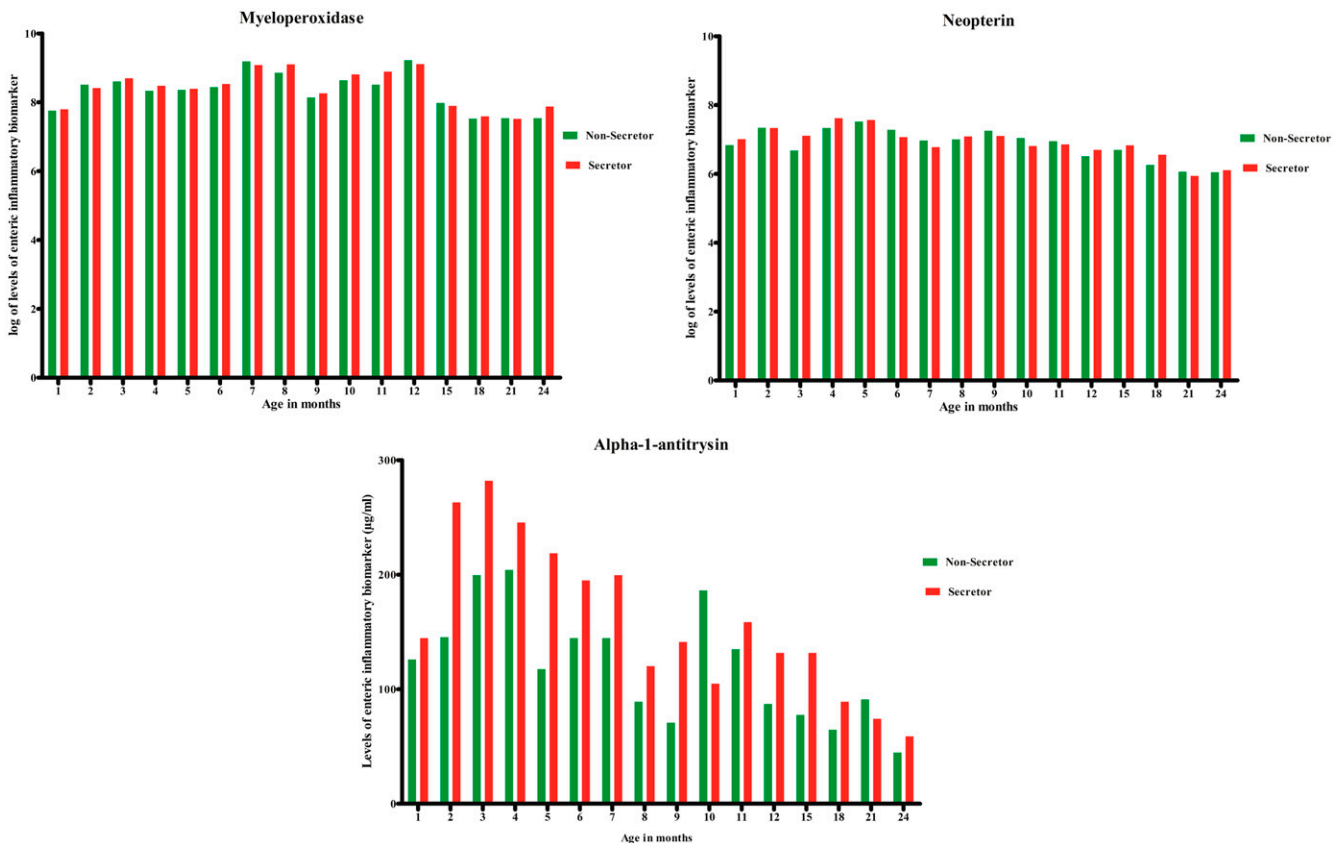


FIGURE 1. Distribution of the enteric inflammatory biomarkers between the secretors and nonsecretors from 1 to 24 months of age. This figure appears in color at [www.ajtmh.org](http://www.ajtmh.org).

TABLE 3  
Association of enteric inflammation with the secretor status of the participants enrolled at the MAL-ED Bangladesh site

Explanatory variable	Myceloperoxidase		Neopterin		Alpha-1-antitrypsin		EED score	
	Adjusted $\beta$ -coefficient (95% CI)	P value	Adjusted $\beta$ -coefficient (95% CI)	P value	Adjusted $\beta$ -coefficient (95% CI)	P value	Adjusted $\beta$ -coefficient (95% CI)	P value
Secretor child	0.04 (-0.07, 0.10)	0.21	0.06 (-0.09, 0.20)	0.13	0.11 (0.07, 0.21)	< 0.01	0.32 (0.29, 0.65)	0.05
Female sex	0.06 (-0.07, 0.19)	0.16	-0.08 (-0.07, 0.06)	0.15	0.19 (0.05, 0.22)	0.04	0.27 (0.04, 0.58)	0.09
Age of child in months	-0.03 (-0.04, -0.02)	< 0.01	-0.06 (-0.07, -0.04)	< 0.01	-0.18 (-0.33, -0.13)	< 0.01	-0.11 (-0.13, -0.09)	< 0.01
Birth weight in kg	0.17 (-0.04, 0.38)	0.19	-0.07 (-0.30, 0.16)	0.52	0.29 (-0.14, 0.4)	0.24	0.19 (0.04, 0.27)	0.15
Duration of exclusive breastfeeding in days	-0.01 (-0.14, 0.11)	0.17	-0.2 (-0.4, -0.05)	0.01	0.03 (0.01, 0.05)	< 0.01	-0.06 (-0.09, -0.03)	< 0.01
Maternal age in years	-0.02 (-0.15, 0.10)	0.13	-0.03 (-0.16, 0.11)	0.12	-0.01 (-0.02, 0.05)	0.22	-0.16 (-0.46, 0.02)	0.11
Length-for-age z score at enrollment	0.09 (-0.08, 0.09)	0.14	0.06 (-0.03, 0.15)	0.18	0.07 (0.03, 0.14)	0.04	0.17 (-0.03, 0.37)	0.09
Detection of Norovirus genogroup II	0.16 (0.03, 0.36)	0.05	-0.05 (-0.24, 0.13)	0.12	0.04 (-0.06, 0.16)	0.10	0.23 (-0.08, 0.55)	0.15
Detection of Rotavirus	-0.025 (-0.26, 0.02)	0.13	0.27 (0.15, 0.55)	0.03	0.13 (0.03, 0.29)	0.01	0.40 (0.86, 0.27)	0.09
Detection of <i>Campylobacter jejuni/coli</i>	0.22 (0.06, 0.39)	< 0.01	-0.03 (-0.19, 0.13)	0.29	0.03 (-0.03, 0.07)	0.19	0.10 (0.05, 0.20)	0.04
Detection of typical EPEC	-0.02 (-0.13, 0.01)	0.10	0.19 (0.04, 0.37)	0.04	-0.07 (-0.17, 0.03)	0.11	0.46 (0.92, 0.40)	0.03
Detection of <i>Cryptosporidium</i> sp.	0.15 (0.13, 0.33)	0.04	0.25 (0.19, 0.53)	< 0.01	0.08 (0.04, 0.18)	0.04	0.57 (0.23, 0.91)	< 0.01
Detection of <i>Giardia</i> sp.	0.36 (0.19, 0.53)	< 0.01	0.10 (-0.29, 0.20)	0.31	0.12 (0.06, 0.24)	0.04	0.37 (0.31, 1.05)	0.03
Detection of <i>Helicobacter pylori</i>	0.20 (-0.14, 0.53)	0.25	0.36 (0.29, 0.74)	< 0.01	0.30 (0.25, 0.54)	0.04	-0.05 (-0.18, 0.29)	0.65
Detection of ETEC	0.21 (0.08, 0.32)	< 0.01	-0.02 (-0.16, 0.12)	0.21	0.12 (0.04, 0.17)	0.02	0.55 (-1.05, 0.89)	0.26

EED = environmental enteric dysfunction.

children by 0.11 units ( $P < 0.01$ ) and 0.32 units ( $P = 0.05$ ), respectively. However, we did not find any significant association between MPO levels and the NEO levels with the secretor-positive status of children, despite having a positive effect size (as represented by the positive  $\beta$ -coefficient) for the associations of MPO and NEO with secretor-positive status of children. On the other hand, we did not find any significant association between secretor-positive status of children with the anthropometric indices of LAZ and WAZ, respectively (Table 4). Nevertheless, we observed a negative effect size (as represented by the negative  $\beta$ -coefficient) for the association between secretor-positive status of children with LAZ and WAZ, respectively, indicating a decreasing trend of LAZ and WAZ among secretor-positive children.

## DISCUSSION

Our study findings show the distribution of secretor status among Bangladeshi children and their mothers and illustrate a significant positive association between childhood enteropathy (as denoted by the EED score) and secretor-positive status of Bangladeshi children. Previous findings have shown that the prevalence of the secretor phenotype varies with different ethnicities, with a prevalence of 70–80% among Caucasians but only 55% among Africans.<sup>46,47</sup> On the other hand, a number of concurrent studies have demonstrated that the prevalence of Lewis-negative individuals, expressing neither Lewis A nor Lewis B and classified as “inconclusive secretor status” is approximately 7%.<sup>48–50</sup> In our study, we found that the prevalence of secretors was approximately 59% among the children and 65% among the mothers. This observation differs from the reports from an observational study conducted among voluntary blood donors in Tamil Nadu, India, where they found that the prevalence of secretors was 75%.<sup>51</sup> In our study, we report higher nonsecretor prevalence among males. In a study in Manipur, India, 10% higher nonsecretor rate was found among males<sup>52</sup> and similar results were found in Rome among asthmatic patients.<sup>53</sup> Additionally, we also report approximately 15% and 10% prevalence of “inconclusive secretor status/Lewis negative” among the children and mothers, respectively. This is in concordance with the findings from the previously mentioned observational study conducted among voluntary blood donors in India, where they reported a 15% prevalence of Lewis-negative individuals.<sup>51</sup>

Moreover, we report that the prevalence of secretor mothers was more among the secretor children and vice versa for nonsecretors (Table 2). This phenomenon may be explained by the presence of Lewis antigens in breast milk,<sup>54</sup> which in turn may act as inducers for the activation of genes responsible for the expression of Lewis antigens in children. In addition, the maternal secretor status influences the distribution and concentration of human milk oligosaccharides (HMOs) expressed in breast milk. These changes in breast milk composition may result in alteration of the microbiome of the child,<sup>55,56</sup> which in turn may lead to a difference in the innate immune system of the child for resistance to enteric infections. The HMOs are modified in secretor mothers' milk making the milk richer in fucosylated oligosaccharides. It is speculated that these modified HMOs can act as decoy receptors serving as anti-adhesive antimicrobials, thus, protecting breastfed babies from enteric infections. Maternal

TABLE 4  
Association of length-for-age and weight-for-age z scores with the secretor status of the participants enrolled at the MAL-ED Bangladesh site

Explanatory variables	Length-for-age z score		Weight-for-age z score	
	Adjusted $\beta$ -coefficient (95% CI)	P value	Adjusted $\beta$ -coefficient (95% CI)	P value
Secretor child	-0.14 (-0.24, 0.11)	0.18	-0.23 (-0.35, 0.07)	0.13
Female sex	0.28 (0.04, 0.51)	0.02	-0.04 (-0.27, 0.25)	0.27
Age of child in months	-0.06 (-0.07, -0.04)	< 0.01	-0.04 (-0.09, -0.03)	< 0.01
Birth weight in kg	0.58 (0.18, 1.17)	0.03	0.64 (0.19, 1.09)	< 0.01
WAMI score	0.36 (0.06, 0.60)	0.05	0.27 (-0.01, 0.48)	0.36
Maternal height in cm	0.13 (-0.02, 0.40)	0.17	0.18 (-0.01, 0.24)	0.23
Maternal weight in kg	0.19 (0.7, 0.29)	0.02	0.16 (0.12, 0.26)	< 0.01
Length-for-age z score at enrollment	0.50 (0.30, 0.69)	< 0.01	0.07 (-0.12, 0.26)	0.25
Detection of EAEC	-0.34 (-0.69, -0.11)	0.04	-0.30 (-0.38, -0.32)	< 0.01
Detection of <i>Campylobacter jejuni/coli</i>	0.021 (-0.02, 0.06)	0.19	0.02 (-0.08, 0.05)	0.22
Detection of ST-ETEC	-0.12 (-0.26, -0.06)	0.03	-0.36 (-0.54, -0.21)	< 0.01
Detection of <i>Shigella</i> sp.	-0.11 (-0.41, -0.06)	0.02	-0.37 (-0.71, -0.14)	< 0.01
Detection of Norovirus genogroup I	0.05 (-0.03, 0.07)	0.21	-0.39 (-0.41, -0.32)	< 0.01
Detection of <i>Giardia</i> sp.	-0.14 (-0.49, -0.08)	0.01	0.11 (-0.03, 0.15)	0.15
Secretor mother	-0.21 (-0.38, -0.07)	0.02	-0.09 (-0.41, 0.02)	0.10

WAMI = Water, sanitation, hygiene, Asset, Maternal education, and Income index.

secretor status may also act through its effect on a child's gut microbiota. The digestion-resistant HMOs serve as metabolic substrates for intestinal microbiota and help shape microbiota composition.<sup>57-59</sup>

Children who are secretors present Lewis antigens on the lining of their gut epithelium, which, in turn, acts as attachment sites or "decoys" for the binding of a plethora of enteropathogens, before host cell invasion and pathogenesis.<sup>60-63</sup> A previous study conducted in Burkina Faso reported that nonsecretors were protected against infections by certain genotypes of rotavirus.<sup>64</sup> Recent findings from a multicenter study showed strong associations between Lewis group antigens and enteric infection burden for some specific enteropathogens.<sup>37</sup> Henceforth, secretor children are more susceptible to enteric inflammation.<sup>60-63</sup> This is evidenced by our study findings, whereby we demonstrate a significant positive association between secretor-positive status and AAT and EED score (Table 3). The respective effect size for the association of the outcome variable of AAT and EED score and the enteropathogens that have been previously reported to be associated with the secretor status of children have also been shown in Table 3. Our results thus indicate that secretor-positive children are at greater risk of development of enteric inflammation and concurrent protein loss, which may reflect the negative effect size for the association of secretor-positive status of children with their LAZ and WAZ, respectively, despite not being statistically significant (Table 4).

The strengths of our study involve the use of a sophisticated and extensively validated multiplex quantitative PCR (qPCR) system for the detection of a number of enteropathogens from a single sample and that we were able to include both the children and their mothers for the assessment of secretor status and its association with childhood enteropathy. However, we were not able to assess the secretor status for the mothers of all the children, whom we had enrolled in our study. We were unable to do genotyping for the individuals (both children and mothers) for whom we were unable to determine the secretor status by using the in-house ELISA method. Henceforth, the possibility of novel polymorphisms existing in the FUT-2 and FUT-3 genes of these individuals with "inconclusive secretor status" is thus very much

pertinent with regard to the limitations of our study. In addition, small intestinal endoscopy, the gold standard for diagnosis of EED, was not performed and so we do not have any pertinent data regarding any possible molecular or immunological aberrations in the upper GI biopsy specimens of the study children.

## CONCLUSION

From our study, we report that the prevalence of secretors among Bangladesh children was approximately 59% and that among their mothers was 65%. The percentage of males among the nonsecretors was greater than that of the females in the nonsecretor group. Our study findings show a potential positive association between the secretor-positive status of Bangladeshi children with childhood enteropathy. Because enteropathy is considered to be an integral factor for childhood growth failure. Our findings thus implicate toward the genetic factor of secretor status of children being associated being linked with childhood growth faltering, through increased susceptibility to distinct enteropathogens and the consequent development of enteric inflammation and enteropathy among children. Thus, our study findings provide unprecedented cues responsible for enteropathy among children and the consequent growth failure. However, our findings are only applicable to settings in Bangladesh and thus need to be validated in several other similar settings, to establish a possible relationship between the secretor status of children with enteropathy and eventual childhood growth failure.

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