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Fecal secretory immunoglobulin A (sIgA) levels in Hirschsprung's disease and healthy controls: a preliminary study

Riana Pauline Tamba*[1], Juwita Cresti Rahmaania[2], Yusra Yusra[3], Valerie Afiyah Marzuki[4]

[1]*Division of Pediatric Surgery, Department of Surgery, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia*
[2]*Department of Surgery, Dr. Cipto Mangunkusumo Hospital, Jakarta, Indonesia*
[3]*Department of Clinical Pathology, Dr. Cipto Mangunkusumo Hospital, Jakarta, Indonesia,*
[4]*Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia*

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***Corresponding Author:** Riana Pauline Tamba, rianatamba@gmail.com

ABSTRACT

Aims: Hirschsprung's disease (HD) is a congenital neurocristopathy characterized by distal colonic aganglionosis and is associated with an increased risk of enterocolitis and other postoperative complications. Impaired mucosal immunity, particularly alterations in secretory immunoglobulin A (sIgA), has been proposed as one of the contributing mechanisms. This study aimed to compare fecal sIgA levels between children with HD without enterocolitis and healthy controls.

Methods: This exploratory cross-sectional study enrolled pediatric patients aged 2 months to <18 years between September and December 2024 from two referral hospitals and one primary health center. A total of 32 subjects were included: 16 children with histopathologically confirmed HD without enterocolitis and 16 healthy controls. Control participants had no congenital gastrointestinal abnormalities or recent gastrointestinal symptoms and showed normal findings on physical examination, routine stool analysis, and fecal occult blood testing. Fecal samples were analyzed using enzyme-linked immunosorbent assay to quantify sIgA levels. Data normality was assessed using the Shapiro–Wilk test, and group comparisons were performed using the Mann–Whitney U test.

Results: The median fecal sIgA level was 1551.56 µg/ml (27.69-35988.75) in the HD group and 771.87 µg/ml (31.27-11250.52) in controls. Although the median value was numerically higher in the HD group, the difference was not statistically significant (p=0.72).

Conclusion: Children with HD without enterocolitis showed a numerically higher median fecal sIgA level than healthy controls, but no statistically significant difference was observed. These findings should be interpreted as exploratory baseline data rather than confirmatory evidence of clinical or mechanistic significance.

Keywords: Hirschsprung's disease, Hirschsprung-associated enterocolitis, secretory immunoglobulin A (sIgA)

INTRODUCTION

Hirschsprung's disease (HD) is a congenital gastrointestinal disorder characterized by the absence of ganglion cells in the distal colon, resulting in functional intestinal obstruction. The estimated global incidence is approximately 1 in 5,000 live births, with a male-to-female ratio of 4:1. Although national incidence data in Indonesia are currently unavailable, extrapolation based on population and birth rate estimates suggests a considerable annual number of affected newborns.[1]

Since the first successful surgery by Swenson and Bill in 1946, surgical management of HD has evolved significantly. These include procedures such as Duhamel, Rehbein, Soave, and Boley techniques. Advances toward single-stage and minimally invasive procedures have reduced hospital stay and improved postoperative quality of life.[2-4] Nevertheless, postoperative complications remain a major concern. Hirschsprung-associated enterocolitis (HAEC) continues to be the most serious complication, with reported early prevalence of 28.73% and late prevalence of 21.37%. Soiling and incontinence are also reported in 20.99% of early and 19.66% of late postoperative cases.[2]

Recent evidence suggests that impaired mucosal immunity may contribute to the development of the complications. Huang et al.[2] demonstrated that low serum immunoglobulin A (IgA) levels were significantly associated with the occurrence of enterocolitis (OR=2.766) and soiling/

incontinence (OR=3.885). Reduced serum IgA is believed to reflect decreased secretory immunoglobulin A (sIgA), which plays a crucial role in intestinal mucosal defense. Lower sIgA levels may weaken gastrointestinal immune protection, facilitating infection, altering the intestinal milieu, and increasing the risk of enterocolitis and incontinence.

Secretory IgA is produced by plasma cells along the intestinal mucosa and functions as a primary immunological barrier by coating microbiota and preventing pathogen adherence. It has been detected in fecal samples of patients with enteritis, where elevated levels are thought to represent a response to infection or inflammation.[5] However, current literature has not specifically examined fecal sIgA expression in patients with HD without enterocolitis. In HD, aganglionosis of the rectum and sigmoid colon that presented in approximately 80% of cases, causes functional obstruction which may promote bacterial translocation and influence sIgA production as part of an immune response.[6]

There is no study specifically evaluating fecal sIgA levels in children with HD without enterocolitis compared with healthy controls. This represents an important gap in understanding baseline mucosal immune status in HD prior to inflammatory complications. Therefore, this study aimed to determine the differences in fecal sIgA levels between children with HD without enterocolitis and healthy controls. The findings of this study are expected to serve as preliminary data for future investigations evaluating sIgA expression in HD.

METHODS

Ethics

Ethical approval was obtained from the Ethics Committee of Cipto Mangunkusumo National Hospital (Date: 11.10.2024, Decision No: KET-1437/UN2.F1/ETIK/PPM.00.02/2024). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. Written informed consent was obtained from parents or legal guardians of all participants prior to enrollment.

Study Design

This study was a descriptive analytic cross-sectional study using primary data collection. The study was conducted between September and December 2024 at the Pediatric Surgery Clinic of Cipto Mangunkusumo National Hospital, Persahabatan General Hospital, and Senen District Primary Health Center, with laboratory analysis performed at the Integrated Laboratory of the Faculty of Medicine, Universitas Indonesia. Subjects were recruited prospectively using a consecutive sampling technique from eligible patients who met the predefined inclusion and exclusion criteria during the study period. Healthy controls were recruited during the same period from the participating outpatient/primary-care setting.

Study Population and Sample Size

The target population consisted of pediatric patients aged 2 months to <18 years. The case group included children diagnosed with HD based on clinical evaluation and histopathological confirmation. The control group consisted of healthy children recruited during the same study period from the participating outpatient/primary-care setting and had no congenital gastrointestinal abnormalities or active gastrointestinal infection. Sample size calculation was performed using Sample Size Calculator version 2.0.4 for comparison of two independent numerical groups with 1:1 allocation, a two-sided alpha of 0.05, and 80% power. Because no prior fecal sIgA data in HD were available at protocol development, previously reported serum IgA data were used as surrogate parameters. The minimum required sample size was 16 subjects per group. Given the use of surrogate assumptions and the wide variability subsequently observed in fecal sIgA values, the present study should be interpreted as an exploratory pilot study.

Inclusion and Exclusion Criteria

Children were included in the HD group if they were aged between 2 months and <18 years, had a confirmed diagnosis of HD based on histopathological findings, had no current or prior history of enterocolitis, and had not undergone definitive pull-through surgery. All patients in the HD group had previously undergone colostomy formation. Children were included in the control group if they were aged between 2 months and <18 years, had no congenital gastrointestinal abnormalities, had no symptoms of gastrointestinal infection within the preceding two weeks, and demonstrated normal findings on physical examination, routine stool analysis, and fecal occult blood testing.

Participants from both groups were excluded if they had known immunoglobulin A deficiency, signs of acute abdomen, severe intestinal obstruction, pneumoperitoneum, or confirmed HAEC. Additional exclusion criteria included long-term immunosuppressive therapy exceeding two months, gastrointestinal infection within two weeks prior to sample collection, other gastrointestinal disorders such as anorectal malformation, necrotizing enterocolitis, neuronal intestinal dysplasia, or acute gastroenteritis, and consumption of Lactobacillus supplements within the previous two weeks. Stool microbiology and viral screening were not performed. Therefore, asymptomatic infection could not be completely excluded.

Data Collection and Variables

Demographic and clinical data were collected through medical records and structured interviews, including age, sex, histopathological confirmation of HD (for the case group), and body weight. Only variables that were systematically collected in both groups were included as formal baseline comparators. Feeding exposure was recorded descriptively, whereas breastfeeding duration, formula composition, nutritional/environmental variables, and colostomy duration were not systematically quantified for valid comparative analysis.

Fecal Sample Collection and Analysis

Fresh fecal samples (approximately 15 grams) were collected without specific time restriction, ensuring that samples were not contaminated with urine. In patients with colostomy, samples were collected directly during stoma output. Samples were transported under appropriate temperature conditions and stored at –20°C until analysis. Fecal sIgA levels were

measured using enzyme-linked immunosorbent assay (ELISA) with the IDK® sIgA ELISA kit (Immundiagnostik AG, Germany). Samples were extracted and diluted to a final dilution of 1:75,000 according to the manufacturer's instructions. All measurements were performed in duplicate. Absorbance was read at 450 nm with reference at 620-690 nm, and concentrations were calculated using a standard calibration curve. Results were expressed in µg/ml.

Statistical Analysis

Data distribution was assessed using the Shapiro–Wilk test. Continuous variables were presented as median (range) because fecal sIgA values were not normally distributed. Gender was compared between groups using Fisher's exact test. Age and other descriptive baseline variables were summarized in **Table 1**, while fecal sIgA levels between groups were compared using the Mann–Whitney U test. A p-value <0.05 was considered statistically significant.

Table 1. Baseline characteristic of the study subjects

Variables	HD (n=16)	Healthy controls (n=16)
Age (month)	24 (7-96)	27 (2-72)
0-12 months old	6 (37.5%)	4 (25%)
>12-24 months old	3 (18.8%)	4 (25%)
>24 months old	7 (43.8%)	8 (50%)
Gender		
Male (%)	12 (75%)	10 (62.5%)
Female (%)	4 (25%)	6 (37.5%)
Body weight (kg)	20.01 (7.5-22.2)	12.55 (4.8-35)

Data are presented as median (min-max) for abnormally distributed numeric variable or n (%) for categoric variable. HD: Hirschsprung's disease, Min: Minimum, Max: Maximum

RESULTS

A total of 32 subjects were included in this study, consisting of 16 children with HD and 16 healthy controls. The baseline characteristics of the study subjects are summarized in **Table 1**. The median age was 24 months (range 7-96) in the HD group and 27 months (range 2-72) in the healthy controls. In the HD group, 6 subjects (37.5%) were 0-12 months, 3 subjects (18.8%) were >12-24 months, and 7 subjects (43.8%) were >24 months. In the healthy controls, 4 subjects (25.0%) were 0-12 months, 4 subjects (25.0%) were >12-24 months, and 8 subjects (50.0%) were >24 months. Male predominance was observed in both groups, with 12 subjects (75.0%) in the HD group and 10 subjects (62.5%) in the healthy controls being male. Female subjects accounted for 4 (25.0%) and 6 (37.5%) in the HD and healthy controls, respectively. The median body weight was 20.01 kg (range 7.5-22.2) in the HD group and 12.55 kg (range 4.8-35) in the healthy controls.

The comparison of fecal sIgA levels between groups is presented in **Table 2**. Data normality testing using the Shapiro–Wilk test demonstrated that fecal sIgA levels were not normally distributed in both groups (p<0.05). Therefore, results were expressed as median (range), and comparisons between groups were performed using the Mann–Whitney U test. The median fecal sIgA level in the HD group was 1551.56 µg/ml (range 27.69-35988.75), whereas the median level in the healthy controls was 771.87 µg/ml (range 31.27-11250.52). Although the median fecal sIgA level was higher in the HD group, the difference between the two groups was not statistically significant based on the Mann–Whitney U test (p=0.72).

Table 2. Comparison of fecal sIgA levels between groups

Variables	HD (n=16)	Healthy controls (n=16)	p value
Fecal sIgA (µg/ml), median (range)	1551.56 (27.69-35988.75)	771.87 (31.27-11250.52)	0.72

Data are presented as mean±SD for normally distributed numeric variable and median (min-max). Statistical test: Mann-Whitney U test. sIgA: Secretory immunoglobulin A, HD: Hirschsprung's disease, SD: Standard deviation, Min: Minimum, Max: Maximum

Table 3 presents the comparison of sIgA levels according to age group and sex between children with HD and healthy controls. In the 0-12 months old group, the median fecal sIgA level was 6271.5 µg/ml (range 95.93-35988.75) in the HD group and 9373.82 µg/ml (range 205.63-10817.06) in the healthy controls, with no statistically significant difference (p=1.00). In the >12-24 months old group, the median fecal sIgA level was 2877.73±3734.98 µg/ml in the HD group and 3888.72±5249 µg/ml in the healthy controls with normally distributed data, which was also not statistically significant (p=1.00). In the >24 months old group, the median fecal sIgA level was 1005.82 µg/ml (range 27.69-24086.25) in the HD group and 365.65 µg/ml (range 33.48-10817.06) in the healthy controls, with no statistically significant difference (p=0.77).

Table 3. Comparison of fecal sIgA levels between variables

Variables	sIgA levels in HD (n=16)	sIgA levels in healthy controls (n=16)	p value
Age			
0-12 months	6271.5 (95.93-35988.75)	9373.82 (205.63-10817.06)	1
>12 months-24 months	2877.73±3734.98	3888.72±5249	1
>24 months	1005.82 (27.69-24086.25)	365.65 (33.48-10817.06)	0.77
Gender			
Female	648.50 (94.47-24086.25)	9373.82 (88.61-10817.06)	0.61
Male	3267.75 (27.69-35988.75)	210.37 (31.27-11250.52)	0.28

Data are presented as mean±SD for normally distributed numeric variable and median (min-max) for abnormally distributed numeric variable or n (%) for categoric variable. Statistical test: Mann-Whitney U test. sIgA: Secretory immunoglobulin A, HD: Hirschsprung's disease, SD: Standard deviation, Min: Minimum, Max: Maximum

Based on gender, the median fecal sIgA level in female subjects was 648.50 µg/ml (range 94.47-24086.25) in the HD group and 9373.82 µg/ml (range 88.61-10817.06) in the healthy controls, with no statistically significant difference (p=0.61). In male subjects, the median fecal sIgA level was 3267.75 µg/ml (range 27.69-35988.75) in the HD group and 210.37 µg/ml (range 31.27-11250.52) in the healthy controls, which was likewise not statistically significant (p=0.28).

DISCUSSION

The main finding of this study was that children with HD without enterocolitis showed a numerically higher median sIgA level than healthy controls, although the difference was

not statistically significant. This finding should therefore be interpreted cautiously as preliminary baseline data rather than evidence of diagnostic utility or a mechanistic role of fecal sIgA in stable HD. Recent literature has increasingly recognized that mucosal immune dysregulation is only one component of the multifactorial pathogenesis of HAEC, together with intestinal dysmotility, microbial dysbiosis, and epithelial barrier dysfunction. Therefore, while the present result may suggest altered mucosal immune activity in HD without overt enterocolitis, it does not establish fecal sIgA as a clinically applicable biomarker and should instead be regarded as hypothesis-generating for future larger and better-controlled studies.[6-8]

The baseline characteristics in **Table 1** were interpreted primarily as descriptive context rather than as main explanatory variables for fecal sIgA differences. The median age was broadly similar between groups, and this age profile remains clinically plausible because, although HD is often recognized early in life, recent studies have shown that diagnosis may still occur beyond infancy with considerable variation in age at presentation.[9,10] Male predominance in the HD group was also consistent with recent reports showing that HD remains more frequent in boys than girls.[10] The higher median body weight observed in the HD group should be interpreted cautiously because body weight in children is strongly age-dependent, and nutritional status in HD may vary substantially across patients. A recent multicenter cross-sectional study further showed that undernutrition is not uncommon in children with HD and that weight-related anthropometric indices may worsen with more extensive aganglionosis.[11] Therefore, age, gender, and body weight in the present study should be regarded as background sample characteristics rather than primary determinants of fecal sIgA variation, although residual confounding related to age structure and nutritional status cannot be completely excluded.

Age- and gender-stratified comparisons are presented in **Table 3**. No statistically significant differences were observed between the HD and healthy control groups within any age category or gender stratum (all $p>0.05$). Because subgroup sizes were small and variability was wide, these findings should be interpreted as descriptive only. In a recent cohort study, fecal SIgA levels in infants differed according to birth mode and breastfeeding status at 3 and 12 months, supporting the concept that early-life SIgA maturation is strongly age- and exposure-dependent.[12] Similarly, Granger et al.[13] showed that stool IgA/sIgA in preterm infants followed a developmental trajectory, with earlier detection in breast milk–fed infants and delayed detection in formula-fed infants, further indicating that intestinal IgA expression varies across early postnatal stages. In addition, Li et al.[14] reported that fecal sIgA responses differed between younger (6-12 months) and older (13-24 months) children, suggesting that age may modify intestinal immune readouts even within relatively narrow pediatric age ranges.

In contrast, no clear sex-related pattern was observed in the present study. This is not entirely unexpected, because recent pediatric IgA-related studies have generally highlighted age, feeding, and perinatal exposure as more important determinants of mucosal IgA variation than gender.[13,14] Therefore, the subgroup analysis in **Table 3** should be viewed mainly as descriptive and exploratory, and the absence of significant differences across age and gender categories should not be interpreted as definitive evidence that these factors are unrelated to fecal sIgA in children with HD.

The absence of a statistically significant difference in fecal sIgA levels between groups should be interpreted with caution. First, although the minimum calculated sample size was achieved, the number of subjects remained small for a biomarker study with substantial biological variability, and the very wide range of fecal sIgA values observed in both groups likely reduced the effective statistical power to detect moderate between-group differences. Therefore, the negative result may reflect limited power and a possible type II error rather than a true absence of biological difference. Second, fecal sIgA is influenced by several major determinants of mucosal immunity that were not systematically controlled in the present study, including feeding-related exposures, microbiota-related factors, environmental conditions, asymptomatic infection, and nutritional status. Recent studies have shown that infant gut SIgA levels vary according to breastfeeding and perinatal exposures, while stool IgA expression also differs between maternal milk-fed and formula-fed infants. In parallel, recent HD/HAEC literature continues to support the role of dysbiosis and impaired mucosal defense as part of a multifactorial disease process rather than an isolated immune abnormality. Therefore, the observed numerical difference in fecal sIgA in the present study cannot be attributed solely to HD status and should instead be regarded as a preliminary signal that requires confirmation in larger and better-controlled studies.[8,12,13,15]

Limitations

The present study does not establish direct clinical utility for sIgA in children with HD without enterocolitis. Because the observed difference was not statistically significant and the study was limited by small sample size, wide data variability, and uncontrolled confounding factors, fecal sIgA cannot currently be considered a clinically applicable biomarker in this setting. Another limitation is that stool microbiology and viral screening were not performed in the control group. Although control participants had normal physical examination, routine stool analysis, and fecal occult blood testing, asymptomatic infection could not be completely excluded and may have contributed to variability in fecal sIgA levels. In addition, baseline comparability between groups was not optimal. Differences in body weight and heterogeneity in age distribution may have resulted in residual confounding. Because of the limited sample size, formal adjustment for these variables was not considered feasible, and this should be taken into account when interpreting the findings. Nevertheless, the study provides preliminary baseline data on fecal sIgA expression in stable HD without enterocolitis, a population that remains insufficiently characterized. These findings may therefore be useful for generating hypotheses and informing future studies with larger sample sizes, age-stratified analysis, longitudinal follow-up, and more rigorous control of nutritional, microbiota-related, infectious, and environmental confounders.

CONCLUSION

In this study, fecal sIgA levels were numerically higher in children with HD without enterocolitis than in healthy controls, but no statistically significant difference was observed. Accordingly, the present findings should be interpreted as exploratory baseline data rather than confirmatory evidence of clinical or mechanistic significance. Further studies with larger sample sizes, longitudinal design, age-stratified analysis, and more rigorous control of major confounding variables are needed to clarify the role of fecal sIgA in HD.

ETHICAL DECLARATIONS

Ethics Committee Approval

Ethical approval was obtained from the Ethics Committee of Cipto Mangunkusumo National Hospital (Date: 11.10.2024, Decision No: KET-1437/UN2.F1/ETIK/PPM.00.02/2024).

Informed Consent

Informed consent was obtained from a parent or legal guardian. Where appropriate, age-adjusted assent was also obtained from the child. The inclusion of vulnerable populations in this study adhered to national and international ethical guidelines. Extra care was taken to ensure voluntary participation, understanding, and protection of participant dignity and autonomy.

Peer Review Process

This manuscript was subject to external peer review.

Conflict of Interest

The authors declare no conflicts of interest related to this study.

Financial Disclosure

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Author Contributions

Concept: JCR, RPT; Design: RPT; Control: RPT; Resources: RPT, YY; Materials: RPT, YY; Data Collection and/or Processing: JCR, VAM; Analysis and/or Interpretation: JCR, RPT, YY; Literature Review: VAM; Writing the Article: VAM; Critical Review: All Authors.

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