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Effectiveness of Irrigation Frequency Per Rectal on Intestinal Microbiota Colonies in Hirschsprung Associate Enterocolitis Patients

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ABSTRACT

Introduction. Hirschsprung-associated enterocolitis (HAEC) is the most common life-threatening complication of Hirschsprung's disease. The incidence of HAEC ranges from 6-60% before and 25-37% after surgery. Rectal irrigation with warm 0.9% NaCl has been shown to reduce the incidence of HAEC before and after surgery. Rectal irrigation plays a role in preventing faecal stasis by evacuating feces, as well as reducing bacterial colonies in HAEC patients. This study aims to determine the effectiveness of rectal irrigation with warm 0.9% NaCl on the number of intestinal microbiota colonies in Hirschsprung-associated enterocolitis patients at Dr. Mohammad Hoesin Hospital Palembang.
Methods: A randomized clinical trial without comparison in the form of an add on trial, open label which is an experiment by providing additional measures to a group with the same standard and different treatment. Patients were divided into three groups, including antibiotics ampicillin, ceftriaxone, metronidazole and 0.9% NaCl irrigation 1 time per 24 hours (Group 1), per 12 hours (Group 2), and per 8 hours (Group 3). Comparisons were tested using paired T-test or Wilcoxon's, p-value <0.05 was considered statistically significant.
Results: HAEC patients who received irrigation treatment 1 time, the number of bacterial colonies did not change, in patients with irrigation treatment 2 times the number of colonies decreased by 171,428.57 ± 138,013.11 colonies/ml, while in patients with irrigation treatment 3 times the number of colonies was reduced. colonies decreased by 214.285.71 ± 177.281.05 colonies/ml. There was a significant difference in the number of bacterial colonies before and after irrigation treatment with a frequency of 2 times (p = 0.026) and 3 times (p = 0.027) while with a frequency of 1 time there was no difference in the number of bacterial colonies before and after treatment (p = 1,000).
Conclusion: Rectal irrigation with warm NaCl 0.9% as much as 20 ml/kg with a frequency of 2 times was effective in reducing the number of gut microbiome colonies. There was no significant difference in the number of bacterial colonies between the 2 and 3 times irrigation frequency groups.

1. Introduction

Hirschsprung's disease is the most common cause of intestinal obstruction in neonates. Hirschsprung's disease is a developmental disorder of the enteric nervous system characterized by the absence of ganglion cells in the myenteric plexus of the colon. Because these cells are responsible for normal peristalsis, Hirschsprung's disease patients will develop functional intestinal obstruction at the

aganglionic level.1-4 Hirschsprung-associated enterocolitis (HAEC) is the most common life-threatening complication of Hirschsprung's disease.[5]

The incidence of HAEC ranges from 6-60% before definitive pull-through surgery and 25 - 37% after surgery. All patients with Hirschsprung's disease are at risk for HAEC. HAEC can manifest clinically as flatulence, explosive diarrhea, accompanied by vomiting, fever, lethargy, and even shock.[5-6]

Treatment strategies for acute HAEC include resuscitation, colonic decompression, and antibiotics. Colonic decompression was performed by rectal irrigation using warm 0.9% NaCl. Rectal irrigation with warm 0.9% NaCl has been shown to reduce the incidence of HAEC before and after surgery. Rectal irrigation plays a role in preventing faecal stasis by evacuating feces, as well as reducing bacterial colonies in HAEC patients. In the study of Demehri et al, 36% of patients in the non-irrigated group developed postoperative enterocolitis, compared with 8% of patients in the irrigated group.[7-8]

This study aims to determine the effectiveness of rectal irrigation with warm 0.9% NaCl on the number of intestinal microbiota colonies in Hirschsprung-associated enterocolitis patients at Dr. Mohammad Hoesin Hospital Palembang.

2. Methods

A randomized clinical trial without comparison in the form of an add on trial, open label which is an experiment by providing additional measures to a group with the same standard and different treatment.

Patients were divided into three groups, including antibiotics ampicillin, ceftriaxone, metronidazole and 0.9% NaCl irrigation 1 time per 24 hours (Group 1), per 12 hours (Group 2), and per 8 hours (Group 3).

This study was conducted in the Department of Surgery, Dr. Mohammad Hoesin Palembang. Examination of the rectal swab sample was carried out at the Central Laboratory Installation of Dr. RSUP. Mohammad Hoesin Palembang. Research data collection was carried out on May-August 2021.

Neonates who experienced HAEC where their parents were willing to take part in the study and neonates with complete variable data were included in this study. Those with probiotic therapy, complications, contraindications to antibiotics, and patients who remained bloated despite adequate irrigation (irrigation failure) were excluded from the study.

Patients requiring emergency surgery were included in the drop out group. Descriptive analysis was carried out on all research variable data to obtain the mean and standard deviation. The normality test of the data was carried out with the Shapiro-Wilk test because the number of samples was <50, to determine whether the data were normally distributed or not. The data is normally distributed when $p>0.05$. Homogeneity test was carried out with Levene's Test, to find out whether the variables between groups were homogeneous or not. The data is declared homogeneous if $p> 0.05$. Comparisons were tested using paired T-test or Wilcoxon's, p-value <0.05 was considered statistically significant.

3. Results

Demographic characteristics

In this study, the majority of HAEC patients were infants with a mean age of 19.05 ± 27.8 months (age range 0 months to 98 months) and the highest gender was male (57, 1%).

Table 1. Demographic characteristics

	n	%
Age, Mean ± Std (min-max) Month	19, 05 ± 27,83 (0 – 98 months)	
Age Classification		
Neonatus (1-28 days)	1	4,8
Infant (28 days – 1 year)	14	66,7
Toddler (1-3 years)	1	4,8
Childhood (3 – 11 years)	5	23,8
Sex		
Male	12	57,1
Female	9	42,9
Grade HAEC		
Possible	0	0
Definite	21	100
Severe	0	0
History *Pull Through*		
Before	16	76,2
After	5	23,8
Nutritional status		
Poor	2	9,5
Under	5	23,8
Good	14	66,7
Comoebid		
Yes	6	28,6
Nothing	15	71,4
Genetic		
Yes	0	0
Nothing	21	100

All patients had a definite degree, the majority of patients had not performed pull through surgery (76.2%). In addition, the majority of HAEC patients had good nutrition (66.7%) and only 2 (9.5%) had poor nutrition. There were 6 patients (28.6%) who had comorbidities but no patient had a genetic history (Table 1).

Comparison of demographic characteristics by irrigation frequency

In this study, statistical analysis showed that there were no differences in the classification of age (p = 0.194), gender (p = 0.558), HAEC degree, surgery history (p = 0.159), nutritional status (p = 0.226), comorbidities (p = 0.226). = 1,000), and genetic history between the three irrigation frequencies. It can be concluded that the three groups are homogeneous and worth comparing (Table 2).

Table 2. Demographic characteristics by irrigation frequency

	Frequency			P value
	1 times	2 times	3 times	
Age Classification				
Neonatus (1-28 d)	0 (0,0)	0 (0,0)	1 (14,3)	
Infant (28 d – 1 yr)	7 (100,0)	3 (42,9)	4 (57,1)	0,194
Toddler (1-3 yrs)	0 (0,0)	1 (14,3)	0 (0,0)	
Childhood (3–11 yrs)	0 (0,0)	3 (42,9)	2 (28,6)	
Sex				
Male	4 (57,1)	5 (71,4)	3 (42,9)	0,558
Female	3 (42,9)	2 (28,6)	4 (57,1)	
Grade HAEC				
Possible	0 (0,0)	0 (0,0)	0 (0,0)	-
Definite	7 (100,0)	7 (100,0)	7 (100,0)	
Severe	0 (0,0)	0 (0,0)	0 (0,0)	
History *Pull Through*				
Before	7 (100,0)	5 (71,4)	4 (57,1)	0,159
After	0 (0,0)	2 (28,6)	3 (42,9)	
Nutritional status				
Poor	0 (0,0)	1 (14,3)	1 (14,3)	0,226
Under	0 (0,0)	3 (42,9)	2 (28,6)	
Good	7 (100,0)	3 (42,9)	4 (57,1)	
Comoebid				
Yes	2 (28,6)	2 (28,6)	2 (28,6)	1,000
Nothing	5 (71,4)	5 (71,4)	5 (71,4)	
Genetic				
Yes	0 (0,0)	0 (0,0)	0 (0,0)	-
Nothing	7 (100,0)	7 (100,0)	7 (100,0)	

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Intestinal Microbiota of Hirschsprung Associated Enterocolitis Patients

In this study, the majority of gut microbiota before and after treatment were gram negative (90.5%) where Escherichia coli was found the most followed by Klebsiela pneumonia. Furthermore, the bacteria Citrobacter freundii and Actinectobacter baumanii before treatment and Actinectobacter calcoaceticus were found after treatment in 1 patient sample (4.8%). While gram positive was only found in 2 samples (9.5%). The gram-positive germs found in the samples of this study included Enterococcus faecalis and Enterococcus faecium both before and after treatment of 1 sample (4.8%) (Figure 1).

Intestinal Microbiota Comparison by Irrigation Frequency

In this study, both before and after treatment, the gut microbiota of gram negative was 90.5% and gram positive was 9.5%. Statistical analysis showed that there was no significant difference in the type of gut microbiota (p = 0.575) between the three irrigation frequency groups (Table 3).

Number of Bactery Colonies Before Treatment

Before being given treatment with rectal irrigation, the number of bacterial colonies in each group was examined and compared between groups. From the bivariate analysis, it was found that there were differences in the number of bacterial colonies before treatment, between the 1 and 2 irrigation frequency groups (p = 0.005), and between the 1 and 3 irrigation frequency groups (p = 0.005). However, there was no difference in the number of bacterial colonies before treatment between the irrigation frequency groups 2 times and 3 times (p = 0.755).

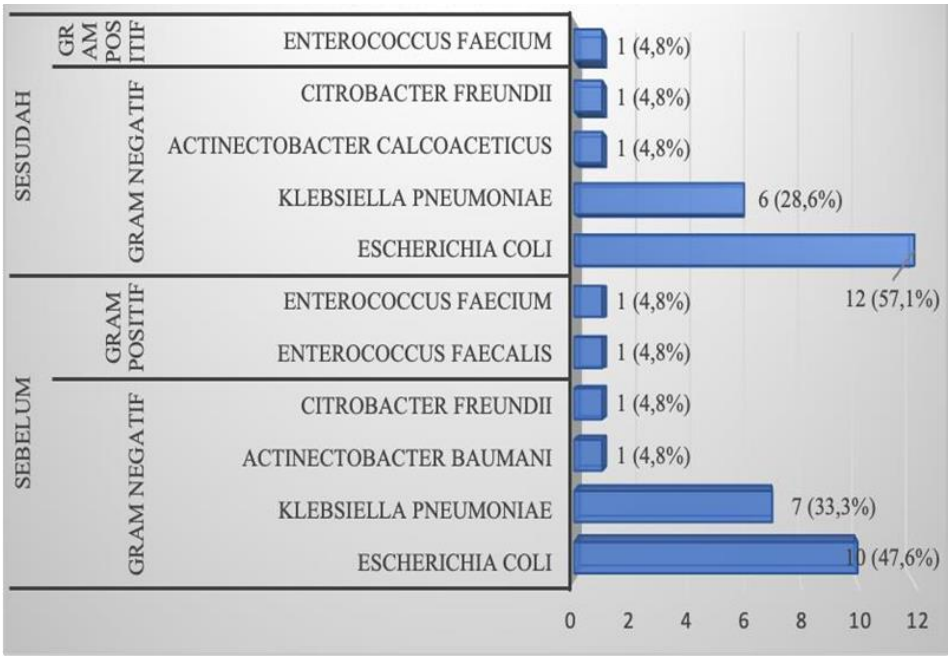

Figure 1. Types of intestinal microbiota of hirschsprung associated enterocolitis patients

After the bivariate test was performed, the analysis continued with the multivariate test. Because the data on the number of bacterial colonies before treatment were not normally distributed, the multivariate test used was Kruskal Wallis. With multivariate analysis using the Kruskall Wallis test, the results showed that there were differences in the number of bacterial colonies between the three groups (p = 0.006) where the largest number of bacterial colonies was in the 3 times irrigation frequency group.

The effectiveness of irrigation frequency on the number of germ colonies between groups after treatment

In this study, in HAEC patients who received irrigation treatment 1 time, the number of bacterial colonies did not change, in patients with irrigation treatment 2 times the number of colonies decreased by 171.428.57 ± 138.013.11 colonies/ml while in patients with irrigation treatment 3 times, the number of colonies decreased by 214.285.71 ± 177.281.05 colonies/ml.

Table 3. Characteristics of intestinal microbiota by irrigation frequency

Characteristics	Irrigation Frequency			P value
	1 time	2 times	3 time	
Gut Microbiota				
Gram Positive	1 (14,3)	0 (0,0)	1 (14,3)	0,575
Gram Negative	6 (85,7)	7 (100,0)	6 (85,7)	

Pearson Chi Square, p = 0,05

Table 4. Comparison of the number of germ colonies before treatment

Group	Mean± Std (colony/ml)	Group	Mean± Std (colony/ml)	p-value
Freq 1 time	107.142,86 ± 18.898,22	Freq 2 times	285.714,29 ± 157.359,16	0,005*
		Freq 3 times	314.285,71 ± 177.281,05	0,005*
Freq 2 times	285.714,29 ± 157.359,16	Freq 3 times	314.285,71 ± 177.281,05	0,755**

**Mann Whitney Test, p = 0,05*

*** Independent T Test, p = 0.05*

There was a significant difference in the number of bacterial colonies before and after irrigation treatment with a frequency of 2 times (p = 0.026) and a frequency of 3 times (p = 0.027) while with a frequency of 1 time there was no difference in the number of bacterial colonies before and after treatment (p = 1,000).

Comparison of the effectiveness of irrigation frequency on the number of germ colonies between groups after treatment

Due to the different number of bacterial colonies before treatment, data on the difference in the number of bacterial colonies was used to compare the effectiveness of irrigation frequency to the number of colonies.

Table 5. Conformity test of the number of germ colonies before treatment

Variable	Treatment Group (n = 7/group)	*Mean* ± Std	*P*
Number of Germ Colonies	Freq 1 time	107.142,86 ± 18.898,22	0,006
	Freq 2 times	285.714,29 ± 157.359,16	
	Freq 3 times	314.285,71 ± 177.281,05	

Kruskall Wallis, p = 0.05

Table 6. Effectiveness of irrigation frequency on the number of germ colonies

Frequency	Before (colony/ml)	After (colony/ml)	p value
Freq 1 time	107.142,86 ± 18.898,22	107.142,86 ± 18.898,22	1,000
Freq 2 times	285.714,29 ± 157.359,16	114.285,71 ± 37.796,45	0,026
Freq 3 times	314.285,71 ± 177.281,05	100.000 ± 0,000	0,027

Wilcoxon Test, p =.0,05

From the bivariate analysis, it was found that there were differences in the number of bacterial colonies after irrigation between the 1 and 2 irrigation frequency groups (p = 0.003) and between the 1 and 3 irrigation frequency groups (p = 0.003). However, there was no difference in the number of bacterial colonies after irrigation between the 2 and 3 irrigation frequency groups (p = 0.623).

After the bivariate test was performed, the analysis continued with the multivariate test. Because the data on the number of differences in bacterial colonies were not normally distributed, the multivariate test used was Kruskal Wallis. With multivariate analysis using the Kruskall Wallis test, the results showed that there were differences in the number of bacterial colonies between the three groups (p = 0.004) where the difference in the

number of colonies was the largest in the irrigation frequency group of 3 times.

4. Discussion

Most cases of Hirschsprung's are diagnosed in infants, although some new cases can be diagnosed until adolescence or young adulthood.[1-4] In this study, the majority of HAEC patients were infants (66.7%) with a mean age of 19.05 ± 27.8 months (range 0 months to 98 months). Yulianda et al (2019) reported that 54.5% of HAEC patients were diagnosed after the neonatal age period.[2] While the study of Demehri et al in 2016 found the median age of HAEC patients was 2.5 years which was included in the toodler category.[7]

El Sawaf *et al* (2012) showed that the mean age of HAEC patients diagnosed was approximately 32 months (toodler). The later the diagnosis of Hirschsprung's disease is established and treated, the more likely it is to develop HAEC in the future.[9]

The incidence of Hirschsprung's disease is about 1 in 4400 to 7000 live births, with an average of 1:5000 live births. With the majority of patients are men compared to women with a ratio of 4:1. In Indonesia the incidence of Hirschsprung's disease occurs around 1: 3250. With the majority of patients being male compared to women with a ratio of 4:1.[1-4]

Table 7. Comparison of the difference in the number of germ colonies after treatment

	Treatment Group (n = 7 / group)		*Mean* ± Std	*P*
Freq 1 time	0,000 ± 0,000	Freq 1 time	171.428,57 ± 138.013,11	0,003*
		Freq 2 times	214.285,71 ± 177.281,05	0,003*
Freq 2 times	171.428,57 ± 138.013,11	Freq 3 times	214.285,71 ± 177.281,05	0,623**

**Mann Whitney Test*, p = 0,05
** *Independent T Test*, p = 0.05

Table 8. Conformity test for the difference in the number of germ colonies after treatment

Variable	Treatment Group (n = 7 / group)	*Mean* ± Std	*P*
Difference in the Number of Germ Colonies	Freq 1 time Freq 2 times Freq 3 times	0,000 ± 0,000 171.428,57 ± 138.013, 214.285,71 ± 177.281,05	0,004

In this study, it was found that men had a greater percentage of experiencing HAEC than women but with a smaller ratio of 4: 3. In the study of Pruitt et al in 2020, it was found that the percentage of HAEC incidence was greater in men (76.6 %). Another study conducted by Le-Nguyen et al in 2019 also found that the majority of HAEC patients were male.[10]

Male patients suffer from HAEC more often than women possibly because of the longer aganglionic segment length than women where long segment aganglionosis has been associated with a higher risk of HAEC.[11-12]

Long-segment Hirschsprung's disease, namely aganglionosis proximal to the splenic flexure, can increase risk of HAEC due to colonic segment dysmotility leading to stasis of lumen contents.[38]

The nutritional status of children in this study was determined based on the WHO growth and development curve for children < 5 years and the CDC growth curve for children > 5 years of age. The majority of HAEC patients (66.7%) had good nutritional status and there were 5 patients (23.8%) with poor nutrition and 2 patients (9.5%) who had poor nutritional status. Yulianda *et al* (2019) also found that 54.5% of HAEC patients had a normal BMI and only 1 patient (9%) had an underweight BMI. Likewise, study Witarto *et al* (2020) which reported that 6 out of 10 HAEC patients (60%) had good nutritional status.[2]

All children had definite degree of HAEC and as many as 16 children had no history of Pull Through surgery. In this study, 6 children with HAEC had comorbidities. These comorbidities include Covid-19, anemia, and hyponatremia.

As for genetic history, no patient had a genetic history or other congenital anomalies.

Statistical analysis showed that there were no differences in age, sex, degree of HAEC, nutritional status, surgery history, comorbidities and genetic history between HAEC patients who were to be intervened with rectal irrigation with a frequency of 1, 2 or 3 times. This means that changes in the number of bacterial colonies which are the parameters of the success of rectal irrigation in this study are not influenced by age, sex, degree of HAEC, nutritional status, history of surgery, comorbidities and genetic history, so the three groups deserve to be compared.

When intrauterine the fetus is exposed to metabolic products from the mother's microbiota. At birth, the baby's digestive tract is sterile and there are no bacteria. Microbial composition in early life is strongly influenced by mode of delivery, maternal microbial composition, feeding method, use of antibiotics, and environmental microbiota contact.[14-16]

Colonies of facultative anaerobic species in the infant intestine include *Escherichia coli, Staphylococcus, Bacteroides fragilis*, and *Streptococcus*.[14] Several microbes (Clostridium difficile, Escherichia coli, Rotavirus) are considered as causative agents in HAEC, but no studies have documented a specific pathogen as the etiology.[6]

In this study, the majority of gut microbiota found from culture results were gram negative (90.5%) where the most common bacteria found were Escherichia coli (52%) and then Klebsiela pneumonia (30%). E. coli is the predominant facultative anaerobic flora of the human gastrointestinal tract. Most strains of E. coli are harmless in the lumen of the large intestine. However, more pathogenic strains have been identified and are commonly associated with urinary tract infections, enteric infections, and systemic infections, including bloodstream infections, neonatal meningitis, and pneumonia.[17]

Study conducted by Neuvonem et al showed that, compared with healthy controls, Hirschsprung patients experienced a significant increase in *Escherichia, Pseudomonas, Dialister, Actinomyces, Bacilli,* and *Prevotella* and a decrease in bacteria that are usually abundant, including Bacteroidales, Ruminococcaceae, and Lachnospiraceae. Patients with a history of recurrent HAEC had significantly different numbers compared to healthy controls and patients without HAEC. Lactococcus and Escherichia were significantly increased in patients with HAEC, but only slightly in patients without HAEC.[6,18-19]

Therapy for children who come with a diagnosis of HAEC is resuscitation, gastrointestinal decompression, and antibiotics. Colonic decompression is essential and can generally be accomplished with rectal irrigation. Irrigation per rectal with warm 0.9% NaCl solution (20 mL/kg) using a large rectal tube.[7,20-21]

Rectal irrigation is useful for the prevention and management of HAEC. Routine rectal irrigation can reduce fecal stasis, bacterial overgrowth, thereby reducing colonic distension. Routine irrigation should be instituted immediately, especially if there is a delay in surgical management. In addition to preoperative prevention, rectal irrigation is also effective for the prevention of postoperative HAEC. Irrigation was carried out with warm 0.9% NaCl 20 mL/kg, one to two times a day.[22-23]

In this study, rectal irrigation with a frequency of 2 and 3 times significantly reduced the number of colonies of intestinal microbiota, but the frequency of rectal irrigation 1 time did not show a change in the number of colonies. The difference in the reduction in the number of the largest bacterial colonies was obtained by giving rectal irrigation treatment in HAEC patients with a frequency of 3 times. However, this difference was not significantly different from rectal irrigation with a frequency of 2 times a day.[22]

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