



ROTAVIRUS INFECTION AS ONE OF THE INDUCING FACTORS THAT RESULTS INVAGINATION IN THE CHILDREN

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ABSTRACT

Background: Invagination is a disease that occurs in children whom require emergency action. Factors associated with the occurrence of invagination during this time can't be determined exactly because 95% of the cause of invagination is unknown (idiopathic), 5% for causal and rotavirus is estimated as one of the risk factors for the occurrence of invagination.

Objective: To discover relationship between rotavirus infection as one of the inducing factors that results invagination in children

Methods: After obtaining approval from the Ethics Committee of the Faculty of Medicine, USU, the parents of the patients were given a detailed explanation of the purpose / benefits of the research, and given the inform consent. Stool examination is divided into invagination and diarrhea group. Stool samples examined in the laboratory by PCR to determine rotavirus.

Results: Most type of invagination is Ileo-colica type with 21 patients (60%) followed by ileo-caecal type with 6 patients (17.1%). There is no significant relationship between sex with invagination incidences ($p = 0.620$); age and the incidences of invagination ($p = 0.542$). From the group of patients with invagination, 23 children were results with positive rotavirus type A (65.7%), whereas in the group of patients with diarrhea, the results were 5 children (25%). There is a significant relationship between the incidences of rotavirus infection and the occurrence of invagination ($p = 0.004$).

KEYWORDS : Invagination, Rotavirus, Age, Sex, PCR

INTRODUCTION

Invagination is a disease that occurs in children whom require emergency action. Definite diagnose for invagination in children is hard to be determined because invagination specific sign 'Invagination Triad' not always found when perform anamneses to the parents or at the examination. (Mac Mahon, 1991).

Seventy five percent (75%) case of children with invagination was found at the age under 3 years whereas 40% it's found at the age between 4 and 12 month's. Incident of invagination was predicted to reach 1 from 2.000 children. Male more dominate than female with ratio 3:2 until 2:1 (Hanz-Iko, 2006).

Result of WHO research which publish at 2000 in 3 main city in Indonesia showed number the occurrence of invagination in children at Medan City are 29 cases, found at age 2 months - 2 year and the most founded in children at age under 1 year about 95% with equivalent of male and female 2:1. Whereas in other city as Jakarta and Yogyakarta the number the occurrence of invagination that found each of city about 103 cases (86% children under 1 year) and 35 cases (61% children under 1 year) with equivalent each male and female for 2:1 and 1:1. (WHO, 2002).

Research which performed in RSUP Haji Adamm Malik Medan and RSUD Pirmgadi Medan at 2010 showed that number of children whom diagnosed with invagination about 49 children (Peny, 2010).

At year 2007 performed research in Yogyakarta about cause of diarrhea and the result found 90% diarrhea caused by rotavirus (Yati Soenarto, 2009).

The observation performed in University Childrens Hospital, Basel, Switzerland at 2006 found 25% cases the occurrence of invagination were leaded by diarrhea (Buettcher M.MD, 2006).

Rotavirus was the most significant caused of diarrhea in baby and some animals. Rotavirus clearly infected mature enterositis vili from small bowel (Angel J, 2013).

during this time can't be determined exactly because 95% of the cause of invagination is unknown (idiopathic), 5% for causal and rotavirus is estimated as one of the risk factors for the occurrence of invagination. (Georges MD, 2012).

Rotavirus had been detected at 3 (41%) patient with invagination, eventhough controlled research just view performed, infection of rotaviral cause *lymphadenopathy* and thickness of distal ileal wall that can be lead point the occurrence of invagination. Because of that thing rotaviral was assesed to have associated with the occurrence of invagination in any cases (Julie, 2004).

Rotaviral infected small bowel and as main caused of severe diarrhea in children. Infection of rotaviral found often in children under 1 year, which as high risk factor for suffered invagination (Kelly L, 2006).

Until this time the researchs had been performed wasn't yet showing fixed associated between infection of rotaviral with invagination, except in 1 case report by dr. Nakagomi in Japan at the end of year 1970 (Georges, 2005).

Observation that performed by researcher for 1 year (2008-2009) in any hospitals in Medan City resulted from 10 cases of invagination, 7 case was leaded of diarrhea causing rotaviral. (Iqbal 2009)

The purpose of this research to find there is a relation Rotaviral as one of causing the occurrence of invagination in children.

METHODE OF RESEARCH

The research programme is observational with kind analytic research that will assessed relationship between Rotavirus with the occurrence of invagination in children. Study that use in this research programme is Cross Sectional Study.

The research is Cross Sectional Study, performed in children with suspected invagination compared to children with diarrhea. The independent variable in this research is Rotaviral and dependent variable is invagination. The parametric that measuring is gender, age.

The factors associated with the occurrence of invagination

The research had been performed since October 2013-March

2014. The collecting research sample performed in any hospital such as RSUP Haji Adam Malik Medan, RS. Malahayati Medan, RS.Mitra Sejati Medan, RS. Methodist Medan, RS. Santa Elisabeth Medan, RS. Sari Mutiara Medan dan RS. Herna Medan. Population of this research are children age 1 month – 3 month. Population separated into 2 group:

Population I : Children with age 1 month-3 years clinically assessed as children with suspect invagination

Population II: (Control) Children with age 1 month-3 years clinically assessed as children suffered diarrhea Research sample collected with determining of inclusion and exclusion criteria into nominal data form.

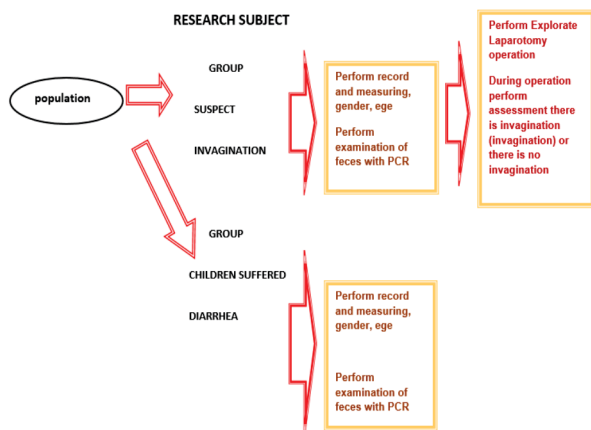
Large of sample for analytic category data, with use formula *Sample Size Determination in Multinomial Logistik Regresi* (Hosmer & Lemeshow, 1998) yaitu : *Rule of thumbs* : Large of sample is 10 times amount of independent variable that researched. Because at this research there were 3 independent variable, so the large of smple: 30 minimum subjek.

Patient with age 1 month – 3 years, children clinically assessed suspected invagination and children with diarrhea, prepared following this research, during operation invagination found caused idiopathic is inclusion criteria in this research. Whereas if research sample there are more than one diagnose at the patient, children with congenital anomaly, denial following this research will be exclusion from the research.

Material and Work Method

1. Obtained license for perform the research from Health Research Committee of North Sumatera and hospital which place this research working.
2. Begining from identification of children suspected invagination (trias invagination) and children suffered diarrhea with age 1 month – 3 year that came to hospital.
3. Obtaining license and permission from the parent or vice person from the children suspected invagination and children with diarrhea that would be subject of the research.
4. Through anamneses which way autoanamnese to the parent or vice person from subject of research, researcher filled medical record that used in the hospital where place the research work on (name, age, gender, etc).
5. Continue for fisisal diagnostic for children suspected invagination and children with diarrhea that would be subject of the research.
6. Perform rotaviral examination with PCR metode to feces of children with invagination and diarrhea.
7. Children suspected invagination perform explore laparotomy operation (invagination operation procedure)

RESEARCH DIRECTION



Examination of feces sample of sufferer invagination and diarrhea with PCR metode

A. Material and Tools that required:

1.Feces is collected from children with invagination and children suffered diarrhea. Than take up to placing (vial) clean and dry. Feces is taken about 1-2 ml or 1-2 mg.

The sample should be brought to laboratory or should be saved in term 2°C until 8°C and must be examine within under 48 hours (2 days). If didn't examine for that time it should be freezed in term -70°C until -80°C.

B. Work Methode:

Preparation of Clinic Sample

Sample Preparation:

- Mix feces sample with water smoothly
- Take about 400 ul of sample (liquid feces sample)
- Vortex for 15 second
- Sentrifuge 12.000 rpm for 10 minute
- Take 140 ul supernatant and filled into 1,5 mL mikro tube.

Extraction of RNA Viruses:

Furthermore, viral RNA was extracted using the QIAmp Viral RNA Mini Kit (Qiagen, Cat Number: 52 904) with the following stages:

1. In the stool supernatant was added 560 ul Buffer AVL
2. Add 6 ul of carrier RNA (Qiagen)
3. Mix by pulse-vortexing for 15 seconds
4. Incubate at room with temperature (15-25 ° C) selama10 minutes
5. centrifugation tube for 15 seconds to remove the pulp from the inside of the lid
6. Add 560 ul of ethanol (96-100%)
7. Mix by pulse-vortexing for 15 seconds
8. centrifugation tube for 15 seconds to remove the pulp from the inside of the lid
9. Apply 500 ul the solution to the QIAamp Mini spin column without wetting the rim
10. centrifugation tube with a speed of 8000 rpm, for 1 minute.
11. QIAamp Put into a 2 ml collection tube clean (Discard the tube containing the filtrate)
12. Repeat steps 10-12 for better results
13. Add 500 ul Buffer Aw1
14. centrifugation tube neck with keceptan 8000 rpm, for 1 minute Place the QIAamp neck 15th player to a 2 ml collection tube clean (Discard the tube containing the filtrate)
16. Add 500 ul Buffer Aw2
17. centrifugation tube neck with a speed of 12000 rpm, for 3 minutes.
18. Take filtrate and place the back of the neck QIAamp Mini to the same tube (Discard the filtrate)
19. centrifugation empty tube neck with a speed of 12000 rpm, for 1 minute
20. Add 50 ul Buffer AVE
21. Incubate at room temperature for 2 minutes
22. centrifugation tube neck with a speed of 12000 rpm, for 2 minutes.

Note: If not directly in RT-PCR, the results of elution can be stored at -800 C for no more.

than 5 days.

(It is recommended to perform RT-PCR reactions on fresh samples)

RT-PCR Rotavirus A, B, and C

RT-PCR Rotavirus A, B, and C

1. Duplex RT-PCR Rotavirus A and C (OneStep RT-PCR Kit, Qiagen; Cat 210210 (100 reaction)

Composition reaction:

Reagents Volume (25 ul)

1. 5x One Step RT-PCR Buffer 5.00
2. 10 mM dNTP Mix 1.00
2. 5x Q Solution 5.00
3. 10 µM primer Beg 90.70
4. 10 µM primer VP7-10.70
5. 10 µM primer G8NS10.70
6. 10 µM primer G8NA20.70
7. Enzyme Mix Poll. 1.00
8. RNA 5.00
9. DEPC – treated water 5.20

Note:

10. All the conditions of the preparation work on the ice
 11. Before RNA was added into the mix RT-PCR, RNA was heated at 92°C for 2 minutes, then immediately put on ice [to remain separate double-stranded RNA], and then enter into the mix RNA RT-PCR)

Primers [Chaimongkol et al., 2012; Yan et al., 2004]:

Beg9: 5'-GGCTTTAAAAGAGAGAATTCGGTCTGG-3'
 VP7-1: 5'-ACTGATCCTGTTGCCATCCTT-3'
 G8NS1: 5'-ATTATGCTCAGACTATCGCCAC-3'
 G8NA2: 5'-GTTTCTGTACTAGCTGGTGAAC-3'

PCR Cycle:

- 50°C, 30 min
- 95°C, 15 min
- 94°C, 30 sec
- 55°C, 30 sec 40 X
- 72°C, 60 sec
- 72°C, 7 min

The results of RT-PCR were analyzed on a 1.5% agarose gel:

Positive Rotavirus A: 395 bp

Positive Rotavirus C: 352 bp

1. RT-PCR Rotavirus B (OneStep RT-PCR Kit, Qiagen; Cat number: 210210 (100 reaks))

Composition reaction:

Reagents Volume (25 ul)

12. 5x One Step RT-PCR Buffer 5.00
13. 10 mM dNTP Mix 1.00
14. 5x Q Solution 5.00
15. 10 µM primer B10.70
16. 10 µM primer B40.70
17. Enzyme Mix Poll. 1.00
18. RNA 5.00
19. DEPC – treated water 6.60

Note:

- All work on the preparation conditions on the ice
- Before RNA added to the mix RT-PCR, RNA was heated at 92°C for 2 minutes, then immediately put on ice [to single-stranded RNA into double-stranded re not], then enter into the mix RNA RT-PCR) Primer [Gouvea et al., 1991; Sen et al., 2000]:

B1: -CTATTCAGTGTGTCGTGAGAGG 5'-3'

B4: 5'-CGTGGCTTTGAAAATTCCTG-3'

PCR cycles:

- 50°C, 30 min
- 95°C, 15 min
- 94°C, 30 sec
- 42°C, 2 min 40X
- 72°C, 1 min

The results of RT-PCR were analyzed on a 1.5% agarose gel:

Positive Rotavirus B: 489 bp

Statistical Analysis:

A Descriptive Analysis Data collection was done with a careful history and charging medical records the study subjects who meet the inclusion and exclusion criteria to a minimum sample size is met. Subject characteristic data obtained from data collection includes: the data for age, sex, results of stool examination (rotavirus +/-) and operating durante findings (invagination +/-) then performed a descriptive analysis by determining the average value, standard deviation and prefalen ratio (PR).

B. Statistics Infrensial

B.1. Univariate analysis

Univariate analysis, the first step is the analysis of the statistical analysis performed on each variable of the study results. In this analysis, namely rotavirus describe all independent variables, age, gender and the dependent variable findings durante exploration laparotomy surgery (invagination +/-)

B.2. Bivariate analysis

In this case use the Chi Square test with 95% significance level. In this analysis describe the two variables are correlated, namely:

1. Children infected / not infected with rotavirus by invagination findings / no invagination durante operation.
2. Age of the child with the findings invagination / no invagination durante child operations.

Gender 3 children with findings invagination / no invagination durante operation.

B.3. Multivariate analysis

Multivariate analysis were conducted on more than two variables, multivariate analysis selected is logistic regression. In this analysis to describe the correlation between:

1 The dependent variable / result (dependent variable) invagination (type invagination) with free variables / causes (independent variables) → rotavirus, age, gender.

2 The results of these observations were tabulated.

RESULTS:

This study was followed by 55 pediatric patients who have met the inclusion criteria. Of the 55 patients obtained 35 pediatric patients with invagination and 20 pediatric patients with diarrhea. The majority of respondents in both groups were boys, there were 25 children (71.4%) with the invagination and 13 children (65%) with diarrhea.

The mean age of patients with invagination was 6.74 months (SD = 5.04) and with diarrhea of 6.35 years (SD = 2.98).

Both groups of respondents are mostly patients with acute diarrhea, 51.4% in the invagination group and 55% in the group of patients with diarrhea.

Based on the type of invagination invagination ileo-known that the type of invagination colica most were 21 patients (60%) followed by ileo-caecal invagination in 6 patients (17.1%).

Table 1 Characteristics of Respondents

Characteristics	Invagination Patient (n=35)	Diarrhea Patient (n=20)	P
Gender n (%)			
Male	25 (71,4)	13 (65)	0,620α
Female	10 (28,6)	7 (35)	

Age, mean (SD), month	6,74 (5,04)	6,35 (2,98)	0,542b
Invagination type, n (%)			
Ileo-ileal	3 (8,6)	-	
Ileo-colica	21 (60)	-	
Ileo-caecal	6 (17,1)	-	
Colo-colica	5 (14,3)	-	

^aChi Square, ^bMann Whitney,

By using the chi square test showed no significant relationship between sex with invagination events (p = 0.620). From the analysis of Mann Whitney test, also found no relationship between age and incidence of invagination (p = 0.542).

Table 2 Relationship of rotavirus infection with Genesis invagination

Rotavirus Type	Invagination Patient (n=35)	Diarrhea Patient (n=20)	p
A	23 (65,7)	5 (25)	0,004
Negatif	12 (34,3)	15 (75)	

In pediatric patients with invagination found as many as 23 children (65.7%) positive type A rotavirus in children with diarrhea while there are 5 children (25%). From the analysis using chi square test, no significant correlation was obtained between rotavirus infection with the invagination.

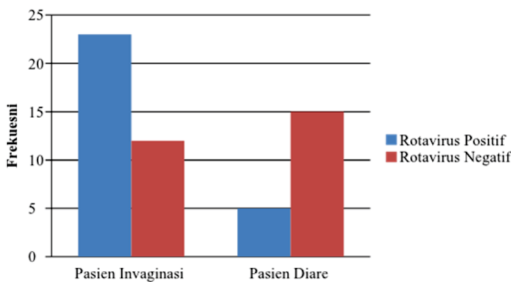


Figure 1 Graph Histogram Number of Patients With Positive Rotavirus Based Presence of invagination

CONCLUSION

1 In patients with invagination found as many as 23 children (65.7%) positive rotavirus type A whereas in patients with diarrhea found as many as 5 children (25%). There is a significant relationship between the incidence of rotavirus infection invagination (p = 0.004).

2 Based on the type of invagination known that ileo-colica invagination invagination is a type with the highest number of 21 patients (60%) followed by ileo-caecal invagination with number 6 patients (17.1%).

3 There is no significant relationship between sex with invagination events (p = 0.620). No relationship was found between age and the incidence of invagination (p = 0.542)

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