International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

Detection Salmonella Typhi RNA from Suspect Typhoid using Reverse Transcription-Polymerase Chain Reaction

Fias[a*], Mochammad Hatta[b], Andi Asadul Islam[c], Rahmawati Minhajat[d], Maryunis Maryunis[e]

[a]*Postgraduate Doctoral Program, Graduate School of Hasanuddin University, Makassar*

[b]*Clinical Microbiology Department, Faculty of Medicine, Hasanuddin University, Makassar*

[c]*Neurosurgery Department, Faculty of Medicine, Hasanuddin University, Makassar*

[d]*Internal Medicine Department, Faculty of Medicine, Hasanuddin University, Makassar*

[e]*Hospital Dr. Wahidin Sudirohusodo Makassar, The Nursing Science Program, Indonesia Muslim University*

[a]*Email: matius_fias@yahoo.com /08124464424*

Abstract

In endemic areas, approximately 90% of enteric fever is typhoid fever. Diagnosis of typhoid fever is difficult to be enforced only on the basis of clinical symptoms alone, because the clinical features of this disease is very varied and generally not typical. Thus the role of the laboratory in assisting the diagnosis is very important. The aim of this research is to detection of Salmonella typhi from suspected typhoid RNA using Reverse Transcription-Polymerase Chain Reaction. The design of this study is cross-sectional analytical survey. The total sample of 101 patients. Data analysis was done using the Chi square test with a confidence level of 95%. All the data were Analyzed by SPSS software, version 21.0 (SPSS, Inc., Chicago, IL). The result showed no association temperature with the results of PCR (p = 0.004), there is a correlation incident dirty tongue with the results of the PCR (p = 0.000), there is a relationship incidence of headache with the results of the PCR (p = 0.027), there is a relationship incidence of dizziness the results of the PCR (p = 0.035), and no association epistaxis events with the results of the PCR (p = 0.024).

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* Corresponding author.

As for the other characteristics: age (p = 0.226), gender (p = 0.819), duration of fever (p = 0.268), fever afternoon (p = 0.579), fever continuously (p = 0.389), cough (p = 1.000), insomnia (p = 0.631), decreased consciousness (p = 1.000), psychosis (p = 1.000), weak (p = 0.314), nausea (p = 1.000), abdominal pain (p = 0.547), anorexia (p = 0.266), constipation (p = 1.000), and diarrhea (p = 0.512) was not associated with the results of PCR. In conclusion, there is a relationship of temperature, dirty tongue, headache, dizziness, and epistaxis with positive PCR test results.

Keywords: PCR; Salmonella typhi; Reverse Transcription-Polymerase Chain Reaction.

1. Introduction

Typhoid fever is a systemic infection caused by Salmonella enteric serovar typhi (S typhi). In endemic areas, approximately 90% of enteric fever is typhoid fever. Annual incidence of typhoid is estimated at about 17 million cases worldwide, 180 / 100,000 person-years in Indonesia [1]. Diagnosis of typhoid fever is difficult to be enforced only on the basis of clinical symptoms alone, because the clinical features of this disease is very varied and generally not typical. Thus the role of the laboratory in assisting the diagnosis is very important. When in the past the laboratory diagnosis of typhoid fever only based on the results of its cause, namely S. typhi isolation from clinical specimens and test widal, then in the last decade there has been a fairly rapid progress in the development of laboratory facilities for diagnosis of typhoid fever [2]. Laboratory tests to establish the diagnosis of typhoid fever are classical methods (culture), serology, and using molecular techniques. However, any method used has advantages and disadvantages of each. DT diagnosis with classical methods, namely by culturing Salmonella typhi from blood samples, stool or urine. Blood cultures have shortcomings, among others in terms of sensitivity, and the length of time spent in culture. Blood cultures have a sensitivity limit is generally caused by the use of antibiotics by patients before the examination, the increase in long (duration) fever, and the small volume of blood used for culture [3].

Serologic testing is still often done that widal technique, has limited the presence of false positives and false negatives, but it has a rather low specificity [4]. Dipstick testing is a method that was developed to meet the needs of laboratory tests are simple and fast. Dipstick testing is a simplification of the ELISA technique that can be used without special equipment [2]. The existing test, developed into a testing tool that is faster and simpler than the dipstick test lateral flow immuno chromatographic test or known lateral flow [5].

Since the discovery of techniques in molecular biology is the use of enzymes to replicate DNA, the polymerase chain reaction or a Polymerase Chain Reaction (PCR) is the only technique that can be used to track the DNA of S. Typhi. Application use of PCR on blood, urine and feces to detect diseases caused by S.typhi DT has been done and has a high sensitivity and specificity [6]. DT is the most common complication of gastrointestinal bleeding (12% in one series) and perforation (3 to 4.6% of inpatients). Approximately 75% of patients found signs of peritonitis. Another complication is neuropsychiatric, cardiovascular, hepatobiliary, genitourinary, hematologic, respiratory, musculoskeletal [1]. Based on the incidence of DT high and complications DT in Indonesia, it is necessary test equipment DT can diagnose quickly and has a value of sensitivity and high specificity, the researchers are interested in doing research "Detection of Salmonella typhi RNA from suspected

typhoid using Reverse Transcription-Polymerase Chain Reaction ".

2. Materials and Methods

2.1. Design

This study is cross-sectional in patients with suspected typhoid fever.

2.2. Sampling

The population in this study are all blood samples from patients who were diagnosed with suspected DT taken from Gatot Subroto Army Hospital, Hospital Mohammad Ridwan Meuraksa Jakarta and Makassar Pelamonia Hospital. This study using purposive sampling technique with a total sample of 101.

a. Inclusion criteria: patients with suspected typhoid fever caused by S. typhi and not get antibiotic treatment. Fever above 37,5ºC and lasts for four days or more. Willing to participate in the study and signed informed consent.

b. Exclusion criteria: patients with fever caused by another type of bacteria, and had never received treatment with antibiotics.

2.3. Analysis of PCR

a. Extraction of nucleic acid

The sample volume of about 100 ul of blood put in 900 mL of solution "L6" consisting of 120g Guanidium thyocianate (GuSCN) (Fluka Chemie AG, Buchs, Switzerland, cat no. 50 990) in 100 ml of 0.1 M Tris-HCl, pH 6.4, 22 ml of 0.2 M Ethylene diamine tetra Acetat (EDTA) pH 8.0 and 2.6g Triton X-100 (Packard, Instrumens) with a final concentration of 50 mM Tris-HCl, 5 M GuSCN, 20 mM EDTA, 0.1% Triton X-100.

Furthermore rotated at a speed of 12,000 rpm. Sediment diatom added 20 mL suspension consisting of 50ml H2O and 500 mL of 32% (w / v) "Celite" ("diatoms") (Jansen Chimica, Beerse, Belgium, 10.846.79). Where 20 mL suspension of this diatom can bind 10 mL of blood, then do "vortex" and centrifuged in a 1.5 ml eppendorf tube with a speed of 12,000 rpm for 15 minutes.

The supernatant was discarded and the sediment was washed with a solution of "L2" consisting of 120 g GuSCN in 100 ml of 0.1 M Tris-HCl, pH 6.4 by adding 1 ml "L2". Furthermore divortex and centrifuged at 12,000 rpm for 15 minutes, then wash repeated 2 times with a solution of "L2", followed by washing with 1 ml of 70% ethanol 2 times and 1 ml acetone.

The C for 10°result is then heated in a water bath at a temperature of 56 minutes and added 60 mL of solution "TE" consisting of 1 mM EDTA in 10 mM Tris-HCl pH 8.0, then do vortex and continued centrifuge at a speed

of 12,000 rpm for 30 seconds, then incubated in the oven for 10 minutes at a temperature of 56oC. Then do the vortex and centrifuge again for 30 seconds at a speed of 12,000 rpm and the supernatant was taken. Supernatant from this process will result nucleotide extraction and stored at -80 ° C prior to PCR analysis.

b. The workings of Realtime PCR to determine RNA gene of S. Typhi

Detecting S.typhi gene using specific primers forward: F: gcgcgaattcagcttttaattaccggtggatgtggcttcc and Reverse: gcgcgaattcgccgtactgcctcaagtaaatttaaagttc. PCR Protocols:

DNA replication is done with the cycle of 94 ° C for 3 minutes, was repeated 38 times cycle Green RT-PCR using qRT-PCR master mix kit, one stage.

This protocol is optimized for instrument MX4000. Protocol adjusted using the instrument by changing the dye dilution based on the instruction manual and follow the instrument factory recommended for the program cycle RT-PCR. The PCR

c. Electrophoresis Gel

Each 10 mL of the amplification product was mixed with 5 mL of solution loading. Once well mixed each put in a 2% agarose gel wells are submerged in a tank containing TBE buffer. Furthermore, the electrophoresis is run for 1 hour at a constant voltage of 75 volts.

After 1 hour, stopped by gel electrophoresis was appointed to be observed under UV light. A positive result if there is a tape RNA and negative if there is no RNA band on the gel. S.typhi RNA PCR product is of 329 bp

2.4. Research Ethics

Permit implementation of the study was obtained from the Ethics Committee FK UH Makassar. After that, the researcher explained to the respondent about the objectives, the benefits of research and collection procedures data. Researcher asked respondents signed informed consent as an endorsement willing to become respondents.

2.5. Analysis of Data

The statistical test used Chi Square with a degree of confidence of 95% and the value of $\alpha \leq 0.05$.Semua Data were analyzed using SPSS version 21.0 (SPSS, Inc., Chicago, IL).

3. Results

The average age of 24.55 ± 17.13 years old respondents, more than half were female (50.5%), the average temperature is 37.41 ± 3,90C, long fever is on average 4.78 ± 2, 25 days, dirty tongue (33.7%), fever in the afternoon (79.2%), persistent fever (49.5%), headache (59.4%), dizziness (71.3%) , cough (29.7%), insomnia (8.9%), epistaxis (2.0%), decreased consciousness (2.0%), psychosis (0%), weakness (20.8%), nausea (79.2%), abdominal pain (52.5%), anorexia (40.6%), constipation (11.9%), diarrhea (20.8%) (Table1).

Table 1: Characteristics of suspected typhoid Patients (n = 101)

Characteristics	**n**	**%**	**Characteristics**	**n**	**%**
Age (year)	Mean	SD	Insomnia		
	24,55	17,13	Positive	9	8,9
			Negative	92	91,1
Temperature (^{o}C)	Mean	SD	Epistaksis		
	37,41	3,9	Positive	2	2,0
			Negative	99	98,0
Fever period (day)	Mean	SD	Decrease consciousness		
	4,78	2,25	Positive	2	2,0
			Negative	99	98,0
Sex			Psikosis		
Male	50	49,5	Positive	0	0,0
Female	51	50,5	Negative	101	100,0
Coated tongue			Weak		
Positive	34	33,7	Positive	21	20,8
Negative	67	66,3	Negative	80	79,2
Fever in afternoon			Nausea		
Positive	80	79,2	Positive	80	79,2
Negative	21	20,8	Negative	21	20,8
Continous fever			Stomach ache		
Positive	50	49,5	Positive	53	52,5
Negative	51	50,5	Negative	48	47,5
Head ache			Anoreksia		

Positive	60	59,4	Positive	41	40,6
Negative	41	40,6	Negative	60	60,4
Dizzy			Konstipasi		
Positice	72	71,3	Positive	12	11,9
Negative	29	28,7	Negative	89	88,1
Caught			Diarrhea		
Positive	30	29,7	Positive	21	20,8
Negative	71	70,3	Negative	80	79,2

Characteristics of respondents based on the results of the PCR was found that there is a connection temperature with the results of PCR (p = 0.004), there is a correlation incident dirty tongue with the results of the PCR (p = 0.000), there is a relationship incidence of headache with the results of the PCR (p = 0.027) , there is a relationship with the incidence of dizziness PCR test results (p = 0.035), and no association epistaxis events with the results of the PCR (p = 0.024).

Table 2: The RT-PCR results According to signs / symptoms

Sign/ Simptoms	**PCR**				**p**	**Sign/ Simptoms**	**PCR**				**p**
	Positive		**Negative**				**Positive**		**Negative**		
	n	**%**	**n**	**%**			**n**	**%**	**n**	**%**	
Age (Year)						Insomnia					
< 25 Year	6	37,5	49	57,6	0,226	Positive	2	12,5	7	8,2	0,631
≥ 25 Year	10	62,5	36	42,4		Negative	14	87,5	78	91,8	
Age						Epistaksis					
Male	7	43,8	43	50,6	0,819	Positive	2	12,5	0	0,0	**0,024**
Female	9	56,3	42	49,4		Negative	14	87,5	85	100	
Temperature (^{o}C)						Kesadaran Menurun					
< 37,41	0	0,0	35	41,2	**0,004**	Positive	0	0,0	2	2,4	1,000
≥ 37,41	16	100	50	58,8		Negative	16	100	83	97,6	

Fever period						Psikosis					
< 5 day	12	75,0	48	56,5	0,268	Positive	0	0	0	0	-
≥ 5 day	4	25,0	37	43,5		Negative	16	100	85	100	
Coated Tongue						Weak					
Positive	16	100	18	21,2	**0,000**	Positive	5	31,2	16	18,8	0,314
Negative	0	0,0	67	78,8		Negative	11	68,8	69	81,2	
Afternoon fever						Nausea					
Positive	14	87,5	66	77,6	0,579	Positive	13	81,2	67	78,8	1,000
Negative	2	12,5	19	22,4		Negative	3	18,8	18	21,2	
Continous Fever						Nyeri Perut					
Positive	10	62,5	40	47,1	0,389	Positive	10	62,5	43	50,6	0,547
Negative	6	37,5	45	52,9		Negative	6	37,5	42	49,4	
Headache						Anorexia					
Positive	14	87,5	46	54,1	**0,027**	Positive	9	56,2	32	37,6	0,266
Negative	2	12,5	39	45,9		Negative	7	43,8	53	62,4	
Dizzy						Constipation					
Positive	15	93,8	57	67,1	**0,035**	Positive	2	12,5	10	11,8	1,000
Negative	1	6,3	28	32,9		Negative	14	87,5	75	88,2	
Cough						Diarrhea					
Positive	5	31,2	25	29,4	1,000	Positive	2	12,5	19	22,4	0,512
Negative	11	68,8	60	70,6		Negative	14	87,5	66	77,6	

As for the other characteristics: age (p = 0.226), gender (p = 0.819), duration of fever (p = 0.268), fever afternoon (p = 0.579), fever continuously (p = 0.389), cough (p = 1.000), insomnia (p = 0.631), decreased consciousness (p = 1.000), psychosis (p = 1.000), weak (p = 0.314), nausea (p = 1.000), abdominal pain (p = 0.547), anorexia (p = 0.266), constipation (p = 1.000), and diarrhea (p = 0.512) was not associated with the results of PCR (Table 2).

4. Discussion

In this study, found an average temperature of 37.41 ± typhoid fever patients 3,90C. The results are consistent with previous research that found the average temperature of patients 37,750C [7]. In this study, it was discovered long fever typhoid fever patients an average of 4.78 days. The results are consistent with previous research that found long fever typhoid fever patients an average of 5.33 days [7]. The results of the present study found no correlation with the temperature PCR test results (p = 0.004). The results are consistent with previous research that found the average temperature of patients 37,750C [7]. The results of this study found no relationship incidence of headache (p = 0.027). And the incidence of dizziness (p = 0.035) with the results of PCR. The results are consistent with previous research that found that patients who were diagnosed positive by PCR assay was found 50% had headaches and 80% had abdominal pain (Moore, and his colleagues 2014). While the research conducted by (Adhikari, Rauniyar, Raut, Manandhar, & Gupta, [8] found an incidence of headache in patients with typhoid fever 97.47%, 45.99% abdominal pain. Research conducted by Adhikari, Rauniyar, Raut, Manandhar, & Gupta, 2015 [8] found in patients with typhoid fever was found vomiting 37.55%, 27% constipation, diarrhea 18.99% This study found symptoms of diarrhea in patients with typhoid fever as much as 20.8%. The results are consistent with research Thriemer, and his colleagues 2012 [7] who found the symptoms of diarrhea in patients with typhoid fever as much as 20% [9].

5. Conclusion

There is a relationship of temperature, dirty tongue, headache, dizziness, and epistaxis with positive PCR test results.

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