The sensitivity of PCR screening on *S. typhi* examination D1-5

D. Prihatini[1], K. Tantular[2], Noor Rachman[3], Handojo[1], T. Pang[4]

Abstrak

Latar belakang dilakukannya penelitian ini adalah kesulitan dalam identifikasi S. typhi *sebagai wahana untuk menegakkan diagnosis pasti pada penderita tersangka demam tifoid. Metode diagnostik yang sekarang dilakukan secara rutin di rumah sakit umum maupun swasta seringkali menunjukkan hasil yang meragukan. Tujuan penelitian ini adalah untuk memperoleh informasi tentang reaksi rantai polimerasa (PCR) yang digunakan untuk diagnosis demam tifoid. Penelitian ini khususnya akan mengevaluasi keunggulan tes ini sebagai tes skrining dalam menegakkan diagnosis. Diperkirakan tes PCR ini dapat membantu menetapkan etiologi suatu gejala demam pada daerah endemik maupun non endemik. Tes PCR itu sendiri mempunyai akurasi dan presisi yang tinggi, dan kelebihannya dalam mendeteksi DNA* S. typhi, *dibandingkan dengan pemeriksaan laboratorium lain yang selama ini telah rutin dilakukan. Dalam penelitian ini akan dilihat apakah tes PCR dapat menapis secara tepat adanya bakteri penyebab penyakit infeksi di daerah endemik. Penelitian dilakukan pada dua kelompok populasi. Kelompok pertama adalah penderita dengan diagnosis klinik demam tifoid. Kelompok kedua adalah penderita demam non tifoid yang didiagnosis secara klinik maupun laboratorik. Batasan populasi yang digunakan adalah: semua umur, bayi sampai dengan dewasa dengan demam lebih dari tiga hari. Variabel yang diperhitungkan dalam penelitian ini adalah kapan penderita dirawat di rumah sakit dan antibiotika yang telah diminum. Dua ratus lima puluh empat sampel darah telah diteliti di Rumah Sakit Dr. Soetomo, Surabaya dan di Institute of Advanced Studies, University of Malaysia, Kualalumpur, Malaysia. Hasil penelitian dianalisis secara statistik dengan metode McNemar dan koreksi Yates (p=0,05). Kesimpulan dari penelitian ini menunjukkan bahwa teknik amplifikasi DNA pada tes PCR dapat menapis* S. typhi *dari mikroorganisma lain dengan derajat sensitivitas penapisan lebih dari 10^5.*

Abstract

The background of the study was the difficulties in the detection S. typhi. *This matter may not support the exact diagnosis of the etiology of a suspect fever. Such as the routinely used method of laboratory test at the government private hospitals or others, it's often shown uncertain results of etiology. The objective of this study is to obtain the information on the character of Polymerase Chain reaction (PCR) test that is used in the diagnosis of typhoid fever. The study concerns particularly in the evaluation of the screening characteristics that may support the diagnosis. The basic result of this PCR test is supposed may assist the confirmation of the fever in endemic as well as in non endemic population. Solely the PCR test has a high accuracy and precision result. Also it has a particular advantage in tracing the* S. typhi *DNA, compared to other routinely used laboratorical test. The aim of this screening study is to determine whether this PCR test could screen precisely the microbe that may cause an infectious disease in an endemic area. This investigation has been carried out using two kinds of populations. First, group of patients who clinically suspected as suffering typhoid fever. Second, group of patients who clinically as well as laboratorically suspected suffering a non-typhoid fever. The criterion and condition of the population: any age, from babies until adults suffering fever more than 3 days. The modifier variables that involved in this study were the time the patients admitted to the hospital and the antibiotic that was admitted. The blood 254 samples were examined at Dr. Soetomo Hospital Surabaya and at the Institute of Advanced Studies, University of Malaysia Kualalumpur Malaysia. The findings were statistically analyzed by McNemar and Yates correction (p = 0,05). This study showed that by amplification technique in a PCR test can screen* S. typhi *from other microorganisms, with the sensitive of screening more than $>10^5$.*

INTRODUCTION

The background of the study was due to the difficulties in the detection of *S. typhi* resulting in failure to diagnose the exact etiology of suspected fever. Furthermore, results of routine laboratory test carried out at the goverment, private hospitals or others, often show a doubtful etiology[2]. The objective of this study is to obtain information on the character of Polymerase Chain reaction (PCR) test used in the diagnosis of typhoid fever[3,4]. The study concerns particularly the evaluation of the screening characteristics that may support the diagnosis. The basic result of this PCR test may assist the confirmation of fever in endemic as well as in non endemic population. The PCR test is known to show high accuracy and precision results. Also it has a particular advantage in tracing the *S. typhi* DNA, compared to other routinely used laboratory tests. The aim of this screening study is to determine whether this PCR test could precisely

[1]*Department of Clinical Pathology School of Medicine Dr. Sutomo Hospital Surabaya, Indonesia,*
[2]*Department of Parasitology School of Medicine, Wijayakusuma University Surabaya, Indonesia,*
[3]*Department of Microbiology School of Medicine Airlangga University Surabaya, Indonesia,*
[4]*Institute of Advanced Studies, University of Malaya, Kuala Lumpur, Malaysia.*

screen the microbe that may cause an infectious disease in an endemic area[1].

MATERIAL AND METHOD

This investigation consisted of two kinds of populations. Firstly, a group of patients clinically suspected of typhoid fever such as: fever more 7 days, dirty tongue, obstipation, meteorism, etc and positive *S. typhi* blood culture. Secondly, group of patients whose clinical as well as laboratory examinations suggested a non-typhoid fever (fever more than 7 days, blood culture of other Salmonellae, dengue fever, malaria). Control subjects: healthy persons with negative blood culture and Widal examination. The criterion and condition of the population: any age, from babies until adults suffering fever more than 7 days. The modifier variables involved in this study were the duration of admission to the hospital as well as the admitted antibiotic. Sera from the patients were collected and kept at -20°C until the PCR was done. Master mix contained:[5]

Primer: Omp 3/4
5' CCG TAA CAC CGA CTT CTT-3'
5' CC G TT G CTG ATG TCC TTA CC-3'
25 mM MgCl2
10 x PCR buffer
10 mM dNTP
DNA template, Taq polymerase

PCR conditions: Denaturation 94° C about 1'
Annealing 60° C about 1'
Extension 72° C about 2'

Electrophoresis gel:
The result of PCR, amplicon was read by agarose gel as DNA fragments. The statistical analysis of the findings done by McNemar and Yates correction (p = 0,05).

RESULT

Table 1. PCR results of samples from typhoid and nontyphoid patient

Patients	N	PCR			
		+	%	-	%
typhoid	45	43	95.6	2	4
nontyphoid	39	0	100	39	100

Result show that 95.6% the typhoid with culture positive have positive PCR, and 100% of nontyphoid patients have negative PCR.

The examinations of 254 blood samples were carried out at the Dr. Sutomo Hospital, Surabaya, Indonesia and at the Institute of Advanced Studies, University of Malaysia Kuala Lumpur Malaysia. From 254 samples from patients with typhoid fever, only 45 were positive for *S. typhi* culture, 39 patients were non typhoid fever (other fever like malaria, dengue haemorhagic fever).

Primer OMP 3/4 could amplify the *S. typhi*, but not other nontyphoid DNA. The PCR result was positive only in fever with positive *S. typhi*, the others were negative. Our results showed that this PCR assay has a sensitivity about 95.6% and a specifity of 100% (Figure 1).

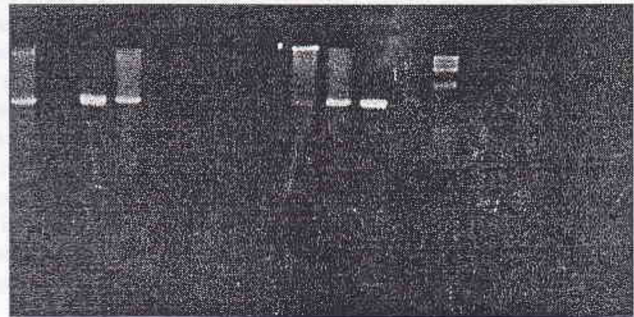

Figure 1. *The results of PCR using Omp 3/4 primer.*
The first lane showed black band of S. typhi control. The next lane shown 5 white bands of amplicon 492 bp of samples with positive S. typhi culture; and the other lanes showed no bands are the amplicon of non typhoid patients. The molecular weight marker is X ∅ 174 Hae III.

DISCUSSION

In this study so far it was shown that the amplification technique in a PCR test was able to screen *S. typhi* from other microorganisms. The screening sensitivity was more than 95%, and the specificity about 100%. Blood cultures were used in this study, because it was impossible to make bone marrow cultures. By using PCR test, the examination can be performed faster than by blood cultures, namely less than 18 hours. It is concluded that PCR test can be used as an alternative method instead of blood culture test if necessary, in urgent cases.

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