

Enzyme linked immunoassay for early diagnosis of Typhoid fever using *Salmonella typhi* outer membrane protein D2-5

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Abstrak

Suatu pemeriksaan Indirect Enzyme Linked Immunoassay (ELISA) dengan antigen yang disiapkan dari *Salmonella typhi* telah digunakan untuk mendeteksi immunoglobulin M antibodi terhadap protein membran luar (OMPs). Sejumlah serum pasien dewasa pada fase akut, minggu pertama yang telah dikonfirmasi dengan kultur sebagai demam tifoid dan serum donor darah telah diperiksa. Didapatkan hasil rata-rata absorbance pada 492 nm pada ketiga kelompok tersebut masing-masing adalah $1,21 \pm 0,59$, $0,15 \pm 0,13$, dan $0,11 \pm 0,08$. Sensitivitas, spesifisitas, nilai duga positif dan nilai duga negatif adalah 81,5%, 100%, 100% dan 90,2%. Dengan menentukan nilai ambang 0,35 dan hasil pemeriksaan ELISA pada serum pasien demam tifoid menunjukkan nilai rata-rata kurang lebih 3,5 kali nilai ambang.

Abstract

An indirect enzyme-linked immunosorbent assay (ELISA) was used to detect immunoglobulin M antibodies against outer membrane protein (OMPs) from *Salmonella typhi* as the antigen. Acute phase, first week, sera from adult patients with culture confirmed typhoid fever, sera from culture negative suspected typhoid fever and sera from healthy blood donors were tested. Sera from the three groups gave a mean absorbance reading at 492 nm, of 1.21 ± 0.59 , 0.15 ± 0.13 and 0.11 ± 0.08 , respectively. The sensitivity, specificity, positive and negative predictive values were 81.5%, 100%, 100% and 90.2%, respectively. The cut off value obtained was 0.35 and in which the ELISA test of typhoid fever patients (Group 1) showed the mean absorbance reading approximately 3.5 times higher.

INTRODUCTION

Typhoid fever is a major problem in developing countries. In Indonesia the number of cases per year is 360-900 per 100,000 inhabitants¹. The serological diagnosis of typhoid fever still relies upon the Widal test, which detects the presence of antibodies to the O (lipopolysaccharide) and H (flagellin) antigens derived from *Salmonella typhi*. According to Schroeder² this test being serological, is non specific, poorly standardized and often confusing. Moreover, it is difficult to interpret in endemic areas, because the antibody titers of the normal population are often not known³ and because of the high background titers among the population⁴. Further, the test must be done 2 times to compare the titers of acute phase and convalescent phase sera. Therefore, in an effort to replace the conventional Widal test, many investigators had already developed a serological diagnosis based on detection of antibodies to *S. typhi* OMPs. OMPs proteins are abundant and exposed on the surface of the cell⁵ and being proteins, have immunogenic properties. Previous studies have indicated that OMPs can

induce immune respons in acute and convalescence phases of typhoid fever⁶. In this study, we examined the ability of OMPs to induce immunoglobulin M (IgM) antibody in the acute phase of typhoid fever. Furthermore, we also compared *Salmonella typhi* IgM antibody detection by ELISA with culture to determine the usefulness of ELISA as an early diagnosis for typhoid fever.

MATERIALS AND METHODS

Human sera

Human sera were divided into 3 groups as follows: group 1, consisted of 27 sera collected from in-patients with typhoid fever, bacteriologically confirmed; group 2, consisted of 46 sera from in-patients suspected typhoid fever, culture negative. The two groups of sera were collected within one week after onset of disease. Group 3, consisted of 70 sera from healthy blood donors.

OMPs preparations (OMPs)

OMPs used in this study was derived from clinical isolate of *S. typhi* and prepared in Laboratory of Microbiology, Faculty of Medicine University of Indonesia using previously described method^{7,8,9}.

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ELISA for the detection of antibodies specific to *Salmonella typhi* OMP

Microtiter plates (96-well, flat bottom, Sarstedt, USA) were coated overnight at 4°C with 100 µl OMPs at a concentration of 5µg/ml protein. Plates were washed 4 times on the next day, with PBS containing 0.1% Tween-20 (PBS-Tween). Blocking was then carried out by the addition of 200 µl PBS, pH 7.4, containing 1% BSA, 0.1% Tween-20, and incubated for one hour at 37°C followed by four washes with PBS-Tween. Primary antibody (human sera) was then added (100µl/well, dilution 1:2000) and the plates were incubated for 1 hour at 37°C. After washed four times with PBS-Tween, 100 µl of Goat Anti-Human IgM-biotin conjugate (Sigma, USA), diluted 1:2000 or 100 µl of Goat Anti-Human IgG-biotin conjugate (1:2000), were added to each well and the plates were incubated for one hour at 37°C. Plates were then washed 4 times with PBS-Tween. Furthermore, 100 µl of Streptavidin-peroxidase labelled (Sigma, USA) diluted 1:2000, was added to each well and the plates were incubated for one hour at 37°C. Plates were washed five times with PBS-Tween before added with 100 µl of the substrate o-phenylenediamine (OPD) dihydrochloride (Sigma, USA) at the concentration of 0.4 mg/ml. The color was allowed to develop for 30 minutes at room temperature and the reaction was stopped with 50 µl 1N H₂SO₄. The absorbance at 492 nm was measured using a Titertek Multiskan MCC340 ELISA reader (America).

RESULTS AND DISCUSSION

Table 1. Comparison of *S. typhi* IgM antibody detection by ELISA with culture

ELISA result	culture	
	positive	negative
IgM positive	22	0
IgM negative	5	46
Sensitivity	81.5%	
Specificity	100 %	
PPV	100 %	
NPV	90.2%	

Results of ELISA test using *S.typhi* OMP preparations as antigen are shown in Figure 1. A significant rise of IgM antibodies in the sera of confirmed typhoid fever patients (group 1) was observed as compared to control groups, i.e. suspected typhoid fever patients (group 2) and healthy blood donors (group

3), with mean absorbance at 492 nm 1.21 ± 0.59 , 0.15 ± 0.13 and 0.11 ± 0.08 respectively. Our results agreed with previous investigators^{8,10}, however, there were slight differences probably due to time variation in sera collection and the dilution of sera. The calculated sensitivity in the ELISA was 81.5%, the specificity 100%, and the positive and negative predictive values were 100% and 90.2% (Table 1), respectively. In this study we also observed higher titers of IgM antibodies in comparison to IgG antibodies in the sera of typhoid fever patients in the first week of illness (Figure 2), so that IgM antibodies might be of more diagnostic significance than detection of IgG. This conclusion was similar to previous investigator¹¹. The cut off value yielded was 0.35 and the ELISA test of typhoid fever patients (group 1) showed a mean absorbance reading of approximately 3.5 times higher.

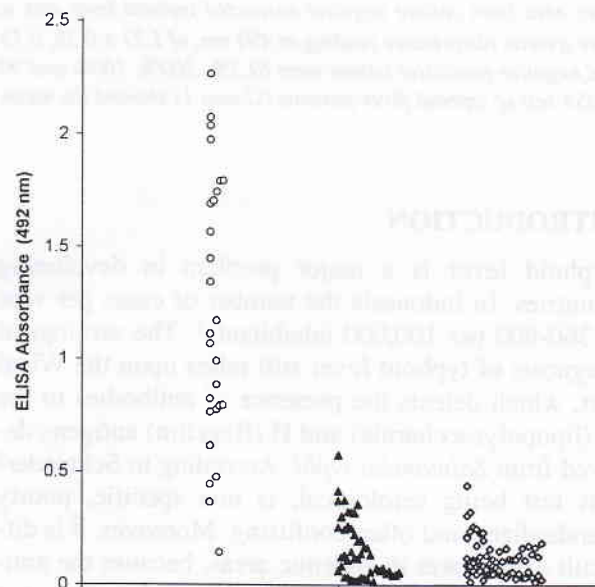


Figure 1. ELISA absorbance readings corresponding to various group of sera.

The first group consisted of 27 patients with confirmed typhoid fever and showed a mean absorbance of 1.21 ± 0.59 . The second group consisted of 46 patients with suspected typhoid fever with a mean absorbance 0.15 ± 0.13 . The third group consisted of 70 healthy blood donors and yielded a mean absorbance of 0.11 ± 0.08 .

In summary, the ELISA using OMP preparations of *S. typhi* as antigen showed high specificity and sensitivity, with value of 100% and 81.5% respectively, in comparison to culture method as the gold standard. We were able to demonstrate the presence of IgM and IgG antibodies in typhoid sera particularly higher

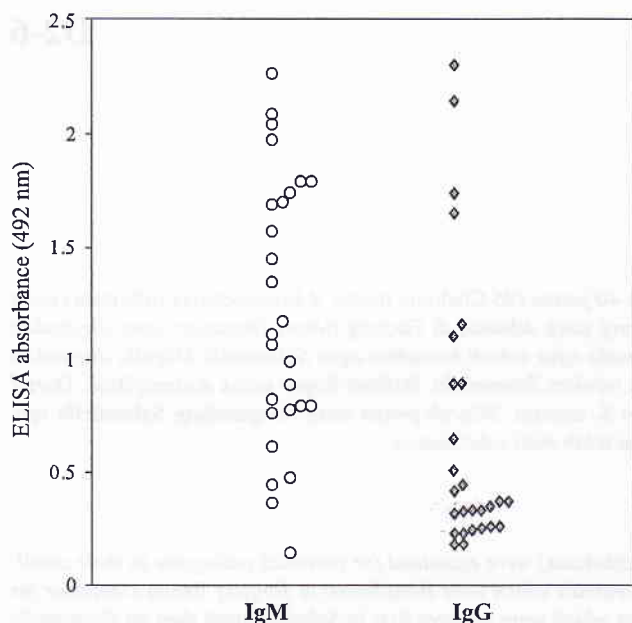


Figure 2. Titer of IgM and IgG antibodies against *Salmonella typhi* OMPs in the sera of Typhoid fever patients.

The mean absorbance of IgM antibodies were higher than IgG antibodies in the sera of typhoid fever patients in the first week of illness. The values were 1.21 ± 0.59 and 0.67 ± 0.12 , respectively.

IgM titer in the first week of illness. Thus, the results suggested that ELISA test might be used for routine early diagnosis of typhoid fever. The cut off value obtained was 0.35 and the mean absorbance for the typhoid fever patients (group1) was approximately 3.5 times higher.

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