Assessment of reactivities of Typhoid fever sera against outer membrane protein preparations from strains of *Salmonella typhi* in Jakarta

S3-2

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Abstrak

Suatu upaya penilaian reaktivitas serum pasien demam tifoid di Jakarta terhadap protein membran luar (Outer Membrane Protein, OMP) Salmonella typhi telah dilakukan. OMP dari 10 isolat klinik S. typhi telah diisolasi dengan sonikasi dan menggunakan buffer yang berisi triton X-100 2 %. Elektroforesis dengan gel Polyacrylamide Sodium Dodecyl Sulfate memperlihatkan protein major Omp C, Omp F dan Omp A dengan berat molekul 38, 37 dan 34 kDa dalam hampir semua sampel. Akan tetapi 3 dari sampel Omp tidak memperlihatkan protein 34 kDa. Meskipun tidak menonjol, polipeptida 20,5 kDa ditemukan pada semua sampel Omp, polipeptida 25 kDa ditemukan pada 4 sampel dan polipeptida 29 kDa ditemukan pada 6 sampel. Selanjutnya polipeptida minor dengan berat molekul yang berkisar antara 20,5 kDa - 139,9 kDa juga ditemukan pada semua sampel. Immunoblotting Omp dengan serum kelinci yang diimunisasi memperlihatkan reaktivitas yang kuat terhadap porin, polipeptida 37, 38 dan 29 kDa. Serum pasien demam tifoid tampaknya berisi IgM yang tidak memperlihatkan reaktivitas silang dengan protein major, tetapi memperlihatkan reaktivitas terhadap polipeptida minor.

Abstract

An attempt was carried out to assess reactivity of sera from patients with typhoid fever against outer membrane protein (OMP) preparations derived from local strain of Salmonella typhi. OMPs from 10 clinical isolates of S. typhi were isolated by sonication and through the use of buffer containing Triton X-100 2%. SDS-PAGE of these OMPs showed six different electrophoretic profiles in which major proteins with molecular weight (MW) 38, 37 and 34 kDa corresponding to OmpC, OmpF and OmpA respectively were present in most of the OMPs. Three isolates of S. typhi, however, did not show the 34 kDa protein. Although not as prominent, polypeptide with MW 20.5 kDa was observed in all OMPs while polypeptide with MW 25 and 29 kDa in 4 and 6 OMP preparations respectively. Immunoblotting of the OMPs with immunised rabbits'sera showed strong reactivity against the porins, the 37, 38 kDa, and the 29 kDA polypeptide. Sera from patients with typhoid fever contained IgM antibodies which did not react with the porins and the OmpA, however, they showed reactivity to the minor polypeptides.

INTRODUCTION

Typhoid fever is one of infectious diseases which gave rise to an important health problem especially in developing countries. In Indonesia the disease is endemic and remains a major public health problem with number of cases per year 360-900 per 100,000 inhabitants in which urban areas have higher incidence than rural areas[1]. Further, case fatality rate in urban areas is higher compared to other countries[2,3].

Salmonella typhi is an etiologic agent and has been shown by many investigators to contain outer membrane proteins (OMPs) which stimulated immune responses in acute and convalescence stages of the disease[4-7]. The proteins induced production of antibodies in significant level so that they were considered of diagnostic value[8-13]. However, those studies suggested the presence of more than one polypeptides that were specific to the microbe.

Analysis of strains of *S. typhi* associated with sporadic cases of typhoid fever and occasional out breaks suggested the existence of genetic diversity among *S. typhi* strains[14,15]. The study indicated that some strains with shared genomic DNA pattern were distributed within Southeast Asia regions i.e. Malaysia, Indonesia and Thailand[15].

In this study we examined characteristics of OMP preparations derived from local *S. typhi* strains and their ability in stimulating immune responses in patients with typhoid fever in Jakarta. If specific OMPs were present, it might be used for diagnostic of the disease in Indonesia as well as in other areas in the Southeast Asia since related strains might present in the region.

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MATERIALS AND METHODS

Bacterial strains

Clinical isolates of *S. typhi* O9,Vi:d were derived from ten patients with typhoid fever in Jakarta. The microbes were cultured and serotyped in our laboratory using standard procedures. *S. typhi* Ty2 were used as a standard strain.

Isolation of outer membrane proteins

S. typhi isolates were cultured overnight in nutrient broth which also contained yeast extract 0.2% and glucose 1.25% at 37°C. Overnight cultures were subcultured in 10^{-3} dilution at 37°C for 6 hours to reach growth at mid logarithmic phase (OD 0.35 at 660nm). OMPs isolation were carried out as described previously[11,16]. Cell suspensions in 10 mM Na_2PO_4 pH 7.2 were disrupted by sonication over ice using Branson ultrasonic sonifier (Branson Ultrasonic Corporation). Sonicated materials were centrifuged at 7000xg for 10 min to precipitate cell debris. Supernatants were collected and centrifuged at 200,000xg for 30 min. Membrane fractions recovered and resuspended in 2% Triton-X 100 and 10 mM Na_2HPO_4, incubated at 37°C for 15 min and recentrifuged. Pellets obtained were washed in 10 mM Na_2HPO_4 and resuspended in sample buffer for SDS PAGE. Protein concentration was measured by standard procedure[17].

SDS-PAGE and Immunoblotting

OMPs were solubilized in sample buffer containing 2% SDS and 0.05% B-mercapto ethanol and electrophoresed on SDS-PAGE containing 12% acrylamide. Electrophoresis was perfomed at 30 mA for 1 h using Miniprotean II apparatus (BioRad). OMPs were electrophoretic transferred to nitrocellulose membranes at 30 mA for 2.5 h using Mini trans-blot electrophoretic transfer cell (BioRad); the blocked nitrocellulose membranes were cut into strips and incubated in immunised rabbits'sera with 1:100 dilution or patients' sera in 1:200 dilution. After washing with PBS-Tween 0.05%, the antibody treated membranes were incubated for 1 h at room temperature with horseradish peroxidase-conjugated goat anti rabbit IgG (1:2500) (BioRad) or anti human IgG (1:500) (BioRad) or IgM (1:800) (Sigma Immuno Chemicals). Immunoreactive bands were visualised by addition of 2mM 4-chloro-2-naphtol and H_2O_2.

Rabbit Immunisation

Two isolates of *S. typhi* in the form of whole cells were used as antigens. Preparation of the antigens and immunisation procedures were carried out as described previously[18].

Human sera

Pooled sera from ten patients with culture confirmed typhoid fever, in acute stage within first week of fever, were analysed for IgM and IgG antibody responses to OMPs. Control sera were obtained from ten healthy blood donors.

RESULTS AND DISCUSSIONS

SDS-PAGE showed major proteins with MW 38 kDa, 37 kDa and 34 kDA corresponded to OmpC, OmpF and OmpA respectively in OMP preparations derived from strandard strain, *S. typhi* Ty2, as well as in most of the OMPs from 10 clinical isolates of *S. typhi*. Three isolates, however, did not show the 34 kDa polypeptide. Minor proteins were observed with molecular sizes ranging from less than 20 kDa to

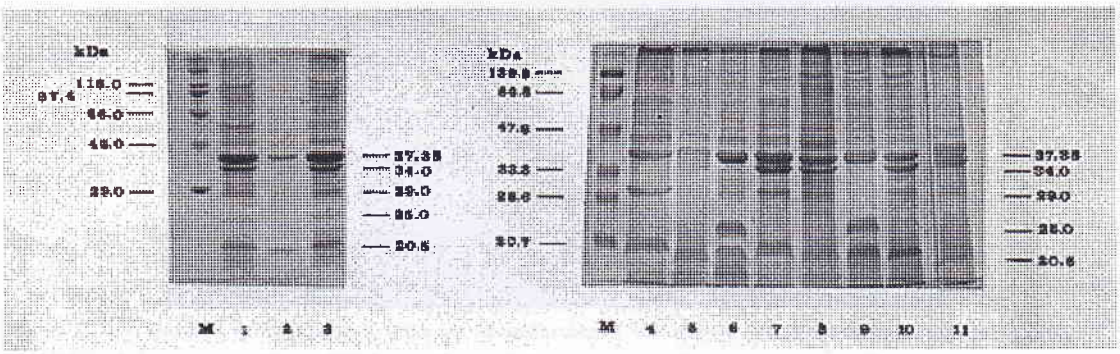

Figure 1. *SDS-PAGE profiles of OMP preparations from 10 clinical isolates of S. typhi.*

Major proteins (38, 37 and 34 kDa) were present in most of the S. typhi OMP preparations (lane 2, 3, 5, 7, 8, 10 and 11) as well as in standard strain, S. typhi Ty 2 (lane 1), except for three S. typhi isolates which did not show the 34 kDa polypeptide (lane 4, 6, 9). Minor polypeptides with MW 20.5 kDa were present in all OMPs, while 29 kDa and 25 kDa polypeptides in 6 OMPs (lane 3,4,7,8,10,11) and in 4 OMPs (lane 3,6,9,11) subsequently. M is protein MW marker.

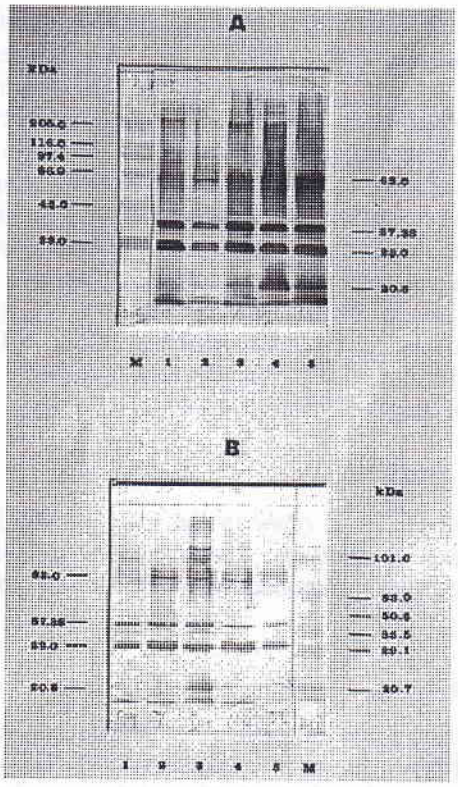

Figure 2. *Immunoblotting of S. typhi OMP preparations with two rabbits' immune sera.*

Sera from rabbits injected with two different S. typhi isolates reacted strongly to polypeptides with MW 38, 37 and 29 kDa from 5 S. typhi OMP preparations tested (Panel A and B lane 1-5). In addition, both sera showed consistency in their reactivity against 63 kDa polypeptide of 3 OMPs (Panel A lane 3,4,5 and Panel B lane 2,3,4). Also they have strong reactivity against 20.5 kDa polypeptide of 1 OMPs (Panel A lane 4) as well as in Panel B lane 3. M is protein MW marker.

around 140 kDa. Polypetide with MW 20.5 kDa was observed in all OMPs, while polypeptides with MW 29 and 25 kDa were observed in 6 and 4 OMPs respectively. One OMPs showed a polypeptide with MW less than 20 kDa (Figure 1). Electrophoretic profiles of major proteins were in agreement with those previously reported[6,11]. In this study, however, we demonstrated that each isolates of *S. typhi* might contain outer membrane proteins which similar in their major proteins but differ in minor ones. We found the presence of six different OMPs' profiles in our *S. typhi* isolates in comparison to *S. typhi Ty2*.

Antigenicity of the OMPs were analysed by immunoblot using rabbits' sera immunised with whole cells *S. typhi*. As shown in Figure 2 A and B, two rabbits' immune sera reacted strongly against the 37 and 38 kDa porins and the 29 kDa protein of all 5 *S. typhi* OMP preparations tested. In addition, both sera reacted with high intensity against other minor proteins i.e. 63 kDa as observed in three of the OMPs, and 20.5 kDa in one of them. The results showed that both major and minor proteins of the outer membrane of *S. typhi* were able to induce immune responses as were reported previously[6], despite each OMPs derived from different isolates might differ in their antigenicity. Our previous study showed that sera from rabbits injected with other enteric bacteria such as *E. coli, S. paratyphi* A, B and C and *S. typhimurium* cross-reacted against *S. typhi* OMPs with MW 37 and 23.5 kDa. Yet, reactivity against *S. typhi* Omps with MW 55 kDa was only observed in sera of rabbits immunised with *E. coli* and *S. paratyphi C*[18].

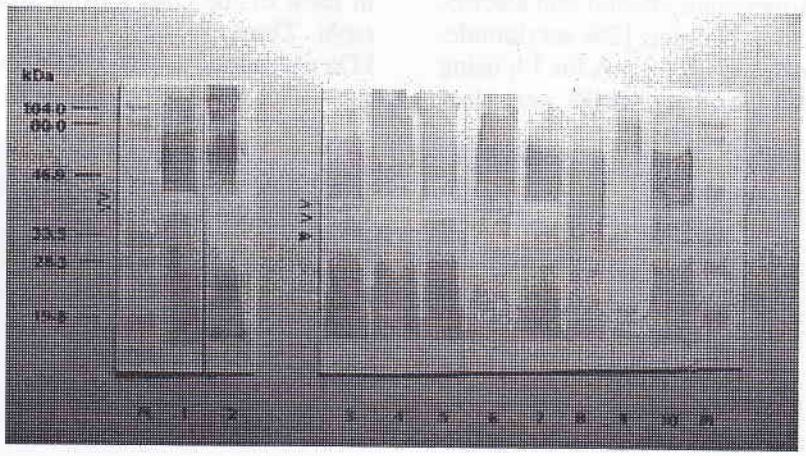

Figure 3. *Detection of IgM antibodies against S. typhi OMP preparations in the sera of typhoid fever patients.*

Acute sera from 10 patients with S. typhi positive cultures were pooled and diluted 1:200, and anti-human IgM HPR-conjugate was diluted 1:800. The sera did not show reactivity against major proteins (38, 37 and 34 kDa). Reactivity against minor polypeptides of the OMPs were observed. Protein band with MW approx. 30 kDa seemed to show stronger reaction (lane 3). Arrow's head indicated porins, and 34 kDa protein, double arrow's head showed 30 kDa protein, M is protein MW marker. Lane 1-10 are OMP preparations from 10 clinical isolates of S. typhi.

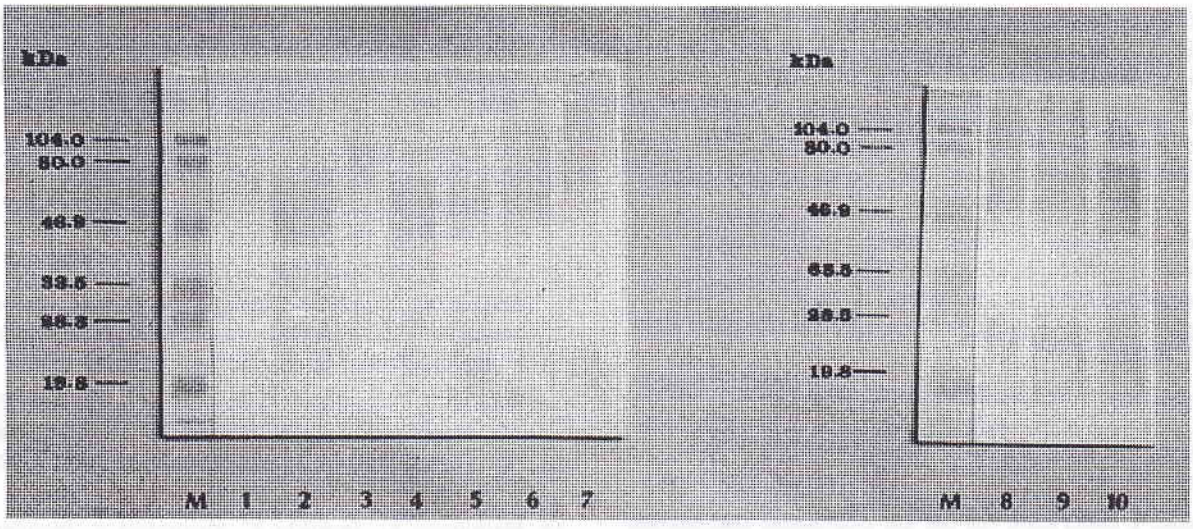

Figure 4. *Detection of IgG antibodies against S. typhi OMP preparations in the sera of typhoid fever patients. Pooled patients' sera were diluted 1:200 and anti-human IgG HRP-conjugate was diluted 1:500. No reactivity against the S. typhi OMP preparations was observed. M is protein MW marker. Lane 1-10 are OMP preparations from 10 clinical isolates of S. typhi.*

To determine the antigenicity of the *S. typhi* OMPs in human, IgM and IgG antibodies against these proteins in the sera of typhoid fever patients were examined by immunoblotting. The results were as follows: pooled patients' sera did not react to major proteins of all *S. typhi* Omp preparations used in the study, suggesting neither IgM nor IgG immunoglobulins were present in response to OmpC, OmpF and OmpA (Figure 3 and 4). Some degree of reactivity of IgM antibodies against minor polypeptides present in the OMP preparations were observed (Figure 3). Protein band with MW approx. 30 kDa in one of the OMP preparations seemed to show stronger reaction to the sera (see Figure 3). Moreover, when anti-human IgG HRP-conjugate was used as second antibody in the immunoblotting analysis, no distinctive reactive protein bands were observed (Figure 4). Immunoblotting using pooled control sera showed no IgM or IgG antibody responses against all of the S. typhi OMP preparations (data not shown). Earlier investigations reported the presence of IgM and IgG immunoglobulin responses directed against *S. typhi* porins in the typhoid fever patients' sera[5,6,12,19,20]. Further, minor polypeptides of the OMPs with MW 50, 53 and 56 kDa were found to be immunogenic in human[8,10,12]. Our results, however, did not confirm theirs, this might indicate the possibility of the presence of different antigenic determinants in our OMP preparations, or else the antigenicity of *S. typhi* OMPs has some degree of variability within strains. Moreover, the possible existence of natural variation in the response to *S. typhi* OMPs among human populations should also be considered.

In the present study, we demonstrated that *S. typhi* OMPs are immunogenic in rabbits administered with the bacteria, in that IgG antibodies recognised the porins and other polypeptides of the OMP preparations. In typhoid fever patients, however, primary and secondary immune responses against the porins are not detected, although we observed that the patients have IgM antibodies against minor polypeptides of the *S.typhi* OMPs. In brief, the presence of antibodies against *S. typhi* OMPs might be an indication of infection of the microbe. However, the use of any specific immunodominant polypeptides of the *S. typhi* OMPs for diagnostic of typhoid fever is in need for further study.

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