Imunodiagnostic

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Research Article

Immunoreactivity of 36 kDa Outer Membrane Proteins (OMP) *Salmonella enterica serovar* Typhi as Candidate Immunodiagnostic for Typhoid Fever

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ABSTRACT

A simple, rapid and early diagnostic test for typhoid fever urgently needed for clinicians. It's have been discovered 36 kDa OMP's from Makassar, South Sulawesi, Indonesia. This study was aimed to determine potency of 36 KDa OMP's to react with antibody of typhoid fever patients as candidate immunodiagnostic of typhoid fever. An OMP 's of *S.* Typhi with an apparent molecular mass of 36 kDa that is highly immunogenic, evokes humoral and cell-mediated immune responses, and confers 100% protection to immunized rats against challenge with very high doses of *S.* Typhi has been identified. Further, very efficient clearance of bacteria from the liver of immunized animals was seen. This protein is recognized by the antibodies present in serum of typhoid patients. The agglutination slide indicated that the immunoreactive protein evoked a strong immune response in rats. Serology test by dri-dot resulted OMP can react with serum of typhoid patients (n=25) with sensitivity 100%.

Keywords: 36 kDa OMP's *S.* Typhi, dri-dot, immunodiagnostic, typhoid fever

INTRODUCTION

Typhoid fever remains an important public health priority, particularly in developing countries, with an estimated 16 million new cases annually and 600.000 death [1]. It is caused by *S.* Typhi, which replicates within the cells of reticuloendothelial system [2]. The emergence of multidrug-resistant strains of *Salmonella* with increased virulence, communicability and survivability leading to increased morbidity and mortality has further complicated its management. Currently available vaccines for typhoid fever have less-than-desired efficacy and certain unacceptable side effects, making it pertinent to search for new immunogen suitable for vaccine formulation [3][4].The OMP's of *Salmonella* have been considered possible candidates for conferring protection against typhoid [5]. Over the past year, several *Salmonella* OMPs have been investigated as potential vaccine candidates, virulence factors, and diagnostic antigen and the molecular structure and function of OMPs and their respective genes have been studied. However, only a small number of OMPs have so far been characterized [6]. On previous studies using *S.* Typhi isolates from Makassar with the results that have been reported Salmonella typhi OMP (Outer Membrane Protein) with a molecular weight of about 36 kDa [7]. Then, this protein was purified and used to detect the immunoreactivity OMP to antibody of typhoid protein sera. Good immunodiagnostics can be used widely for various isolates of *S.* Typhi from all over the world, particularly in Indonesia, because as it is known that *S.* Typhi has spread to many regions mainly due to the nature of the easy spread through contaminated water and food were also accompanied with high human mobility, thus need further investigation as a generalization of the test vaccine made from the protein. Then the results are expected to be the first step of typhoid fever vaccine which has high efficacy and effectiveness in inhibiting *S.* Typhi infection and can be used widely in Indonesia, where has a high population mobility, which might be facilitate the spread of typhoid fever. Hence it needed to find protein from local antigen.

Material And Methods

Adaptation of animal

Prior to immunization in animal models of male mice (*Mus musculus*) ICR adaptation needs to be done first. Adaptation is done aims to create an animal model to use is able to adapt mutually to each other.

Hence, in the ongoing immunization of mice ICR male does not interfere with each other and remain in a state of quiet because it has been used to being in the same enclosure 1. At this stage, 8 mice ICR male were obtained from the Laboratory of Biopharmaceutical Faculty of Pharmacy, University of Hasanuddin and divided into two groups: the treatment group and the control group. Each group consists of 4 individuals were placed in cages and left to stand for 7 days indoors animal pets.

Immunization Animals

After going through stage adaptation, then performed immunize mice (*Mus musculus*) using male ICR with OMP and phosphate buffer saline (pbs) was used as a negative control. Before immunization to injection, blood samples from mice ICR male was taken and stored at -20°C until used. Then the mice male ICR injected subcutaneously into the OMP 36 kDa protein on days 0, 7, 21, 28 and day 40. The control group immunized using pbs on the same schedule. Then take the blood at day 50, and the serum was separated and stored at -20°C until used.

Protection test

After 4 weeks immunization, the treatment group of mice (*Mus musculus*) ICR male using OMP 36 kDa protein were further tested with *S.* Typhi bacteria were injected intraperitoneally. Mice ICR male was observed at day 14 after the protection test bacteria, and then note the condition of mice male ICR to see physical changes that occur as well as the number of mice that survive.

Identification of the liver Mice (*Mus musculus*) ICR Male

Furthermore, the identification of the mice (*Mus musculus*) ICR Male who had been challenged with S. Typhi. Liver of Mice male ICR broth was added and then allowed to stand for 2 days. Furthermore, inoculate on selective media and incubated for 24 hours. Observations were made by looking at colonies on the surface of the media. Colonies of bacteria looks slick, shiny and transparent [8]. Isolates were then replanted on selective media Triple Sugar Iron Agar (TSIA) for 24 hours to see their specific *S.* Typhi bacteria that grows with the production of H2S see whether there is any alteration in black and media be lifted.

Widal test (Slide Agglutination)

By using a special pipette for each dilution, following a number of serum was added on top of the circle diameter 27 mm slide: 0.08 ml; 0.04 ml; 0.02 ml; 0.01 ml And 0,005 ml. Antigens have been suspended completely added about 1 drop right on the circle slide. Then mix and evenly to the entire surface of the circle. Slowly and often, shake and rotate the test slide for 1 minute until you see their agglutination. The results obtained are matched with tube agglutination titer in a row 1:32; 1:64; 1: 160; and 1: 320.

Serology test (Dri-Dot)

OMP test with serum using latex principle. Latex used for embedding OMP is dyed deep blue latex beads with a diameter of 0,80 µm Sigma-Aldrich production already in the form of a suspension. Wash latex with carbonate-bicarbonate buffer 1% then add lyophilized OMP with a ratio of 1 mg in 100 ul Latex. Latex mixture and OMP then incubated at 37°C for 6 hours and then blocking with PBS containing 5 mg/ml BSA to block non-specific absorption of other proteins at 37°C for overnight. Dispense 5 ml latex agglutination OMP in the card, and then added serum by the same amount. Mix the serum and latex using a sterile spatula meet the circle of the agglutination card. While shaken, agglutination observed changes on the cards for 2 minutes. The results are then interpreted. Agglutination occurs under 15 seconds is interpreted +4, +3 result if agglutination occurs between 15 to 30 seconds, +2 if agglutination seen in 30 to 45 seconds, +1 if agglutination formed between 45 seconds to 1 minute. Meanwhile, if there is no change in agglutination card over 1 minute interpreted negatively.

Ethical Approval and Informed Consent

Institutional and ethical permission to carry out the study was obtained from the Faculty of Medicine, Universitas Hasanuddin, Makassar, Indonesia. Adult participants and parents/guardians of suspected children provided by informed consent before blood samples were collected.

Results And Discussion

Protection Studies

After 4 weeks were immunized with OMP protein, male mice (*Mus musculus*) ICR then induced with *S.* Typhi bacteria. Injection was injected intraperitoneally. Observations on day 14 by count the number of mice that successfully survive. Results on Table 1.

Table 1. Number of mice (*Mus musculus*) ICR male that survived until day 14 after tested protection with *S.* Typhi bacteria

Protein	Amount (%) Survive (until day 14)
OMP *S.* Typhi	4 (100)
Control	0 (0)

After going through the immunization for 40 days, mice (*Mus musculus*) male ICR challenged with *S.* Typhi bacteria and injection was injected intraperitoneally. This protection test transactions are carried out with the aim to determine the ability of the OMP protein that is used in recognizing the serum of mice and see the number of male ICR mice survived until day 14. OMP *S.* Typhi plays an important role in the growth of colonies, biofilm formation, and disease progression [9]. OMP is also a major virulence factors associated with colonization of *S.* Typhi [10].

Identification of the liver mice (*Mus musculus*) after protection test with *S.* Typhi

Male Mice (*Mus musculus*) ICR which has tested with *S.* Typhi then identified to detect growth of *S.* Typhi on mice's liver, then cultured by in vitro on selective media for *S.* Typhi

Table 2. Results of growth *S.* Typhi on selective media TSIA and MacConkey

Media	Result	
	Positive (*S.* Typhi)	Negative (*S.* Typhi)
MAC	-	v
TSIA	-	v

The results obtained in the protection test with *S.* Typhi bacteria that has been done is the physical condition of mice (*Mus musculus*) ICR males remain in a state of normal and 100% survive the fourth namely mice ICR male remains alive until the 14th day. This shows that the adaptive immune response has been to recognize foreign substances (antigens) that enter the body of mice ICR male having previously been immunized with OMP 36 kDa protein that acts as an antigen. Adaptive immunity is immunity that arise after exposure to an antigen (e.g. an infectious agent) is specific and mediated by antibodies or lymphoid cells [11]. Basically, adaptive immunity is the immune system of a specific nature that have the ability to recognize objects that are considered as new object to itself. A new objects which first appeared in the body immediately recognized by the specific immune system resulting in sensitization of cells of the immune system. When immune system cells are passed back to the same foreign object, then the foreign object the latter will be known sooner, then destroyed [12].

Widal test

Widal test was conducted in order to detect the presence of antibodies against *S.* Typhi in blood serum of mice (*Mus musculus*) ICR male with comparing results among samples of serum prior to immunization, serum samples after immunization and serum samples in early protection test with *S.* Typhi and final of protection test. The results presented in table 3, table 4, table 5, and table 6.

Table 3. Widal test pre-immunization with OMP (week 1)

Sample code	Titer	Result
Fe. Ri	-	Negative
Fe.lf	-	Negative
Ds	-	Negative
Pt	-	Negative

Fe. Ri (femur-right); fe.lf (femur-left); ds (dorsal); pt (posterior)

Table 4. Widal of post-immunization with OMP (week 5)

Sample code	Titer	Result
Fe. Ri	1/64	Positive
Fe.lf	1/64	Positive
Ds	1/160	Positive
Pt	1/160	Positive

Fe. Ri (femur-right); fe.lf (femur-left); ds (dorsal); pt (posterior)

Table 5. Widal of post-protection studies with bacteria *S.* Typhi (day 3 after protection test)

Sample code	Titer	Result
Fe. Ri	1/320	Positive
Fe.lf	1/1320	Positive
Ds	1/640	Positive
Pt	1/640	Positive

Fe. Ri (femur-right); fe.lf (femur-left); ds (dorsal); pt (posterior)

Table 6. Widal of post-protection studies with bacteria *S.* Typhi (day 14 after protection test)

Sample code	Titer	Result
Fe. Ri	1/32	low
Fe.lf	1/32	low
Ds	Sera insufficient	undetectable
Pt	Sera insufficient	undetectable

Fe. Ri (femur-right); fe.lf (femur-left); ds (dorsal); pt (posterior)

Serology test (Dri-dot) with typhoid patient's sera

The 25 sample of typhoid patients were collected then tested using blood culture (gold-standard). Table 7 shown sensitivity and specify of dri-dot (latex) compare with lateral flow (kit for IgM).

Table 7. Comparative evaluation of Dri-dot OMPs and Lateral flow (blood culture as a gold standard)

	No. of positive among positive culture (n=5)	No. of positive among negative culture (n=20)	Sensitivity	Specify
Dri-dot	5	18	21,74	100
LF	5	18	21,74	100

Based on tests that have been carried out widal obtained results presented in Table 3, 4 and 5 which antibody titers in serum of mice (*Mus musculus*) ICR male before being immunized showed negative results in all four existing samples. While the results of the serum samples were immunized showed that serum on the four samples tested positive with the amount of antibody titers in serum samples with Fe-ri code that is 1/64 and serum samples with Pt code that is 1/64 The results shown in serum samples that had been challenged with *S.* Typhi bacteria are positive in the fourth sample with titers in serum samples with code Ds is 1/160 and the serum samples with Pt code that is 1/640. While in some other samples were not obtained specific titer, this is because the samples obtained contained no serum with a predetermined amount so that the titer of the sample could not be detected. According to Wardhani *et al*. [13], Widal test is considered positive when the antibody titer 1/160. The higher the titer the more likely people suffering from typhoid fever. 1/320 titer indicates that the blood samples of patients who used experienced moderate or mild infection. 1/640 titer indicates that the sample of patients experiencing chronic or severe phase and the need for further handling. The higher the serum is used and there granule shows the level of *S.* Typhi bacterial infection.

Conclusion

Based on the results obtained, it proves that the OMP protein 36 kDa immunogen and *S.* Typhi are able to inhibit the growth of the *S.* Typhi bacteria in the body of mice (*Mus musculus*) ICR male so that it could be used as immunodiagnostic for the diagnosis of typhoid fever. One of the variables in this study aims to establish diagnostic. Diagnostic tests are required with a high specificity, although the sensitivity is not too high. This is in accordance with the results of the study on OMP latex with blood culture that is 100% sensitivity, and low specificity of 21,74%. On the other hand, different results on OMP latex with lateral flow are sensitivity of 95,65% and specificity of 50%. The results presented here showed that all culture positive samples were positive on Lateral Flow and Dri-dot.

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