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INDIVIDUAL HUMAN SERA RESPONSE AGAINST PROTEIN EXTRACTS FROM SALIVARY GLAND OF *Aedes aegypti*

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ABSTRACT

The saliva of hematophagous arthropods contains a complex mixture of biologically active proteins. These proteins may modify hemostatic responses and induce both cellular immunity and the production of specific antibodies, and thus influence the transmission of its pathogens from arthropods vector to human host. *Aedes aegypti* is the main vector for transmission of dengue viruses into human. The objective of this study was to examine individual human sera response against protein extracts from salivary gland of *Ae. aegypti* that mediate the infection of dengue viruses. We did a cross reaction test of human sera from healthy people in endemic and non-endemic area, and dengue patients againts SGE of *Ae. aegypti* to distinguish and to identify the immunogenic proteins using Western Blot Analysis. About 15 protein bands of SGE from *Ae. aegypti* ranging from 15 kDa up to 255 kDa were identified on 12% SDS-PAGE. Seven dominant bands were detected, i.e ~255, 56, 42, 31, 27, 26 and 15 kDa. Two immunogenic proteins, as represented by two bands, i.e. ~31 and 56 kDa were found only in samples from people who were previously exposed to mosquitoes bites, and not in people who had not been exposed. Therefore, these immunogenic salivary proteins may serve as indicators for the immune response in human against protein from salivary gland of *Ae. aegypti*.

Keywords: immunogenic proteins, salivary gland, *Aedes aegypti*,

INTRODUCTION

Dengue virus (DV) causes dengue fever and more severe conditions of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). The World Health Organization estimates that there are 50 million dengue infections every year worldwide (Wasinpiyamongkol *et al.*, 2010). DV is transmitted to vertebrate host by mosquito vectors, with *Aedes aegypti* as the main vector and *Aedes albopictus* as secondary vector. The vectors acquire the pathogens by feeding on infected hosts and then transmit them by regurgitation during a subsequent blood feeding (Ader *et al.*, 2004). Blood feeding is required for nutrition, egg development and survival of mosquitoes (Gillespie *et al*, 2000). Mosquito saliva is vital for successful blood feeding because it contains anticoagulant, anti-inflammatory, anti platelet aggregation and immunosuppressive factors (Ribeiro & Francischetti, 2003). Saliva proteins or salivary gland extracts also have antigenic and immunogenic properties, as they can induce an IgG antibody response in individuals living in endemic areas (Remouse *et al.*, 2007; Waitayakul *et al.*, 2006) and in travellers transiently exposed to vectors in tropical areas (Orlandi-Pradines *et al.*, 2007). These proteins can induce allergic reactions such as itchy and red skin (Fontaine *et al.*, 2011). The development of a natural antibody response in people living in endemic area is due to frequent exposure to mosquito saliva (Cornelie *et al.*, 2007). This indicates that the vector bites have a positive effect on the host immune response. These responses can be used as epidemiological markers of vectors exposure and also support the possibility to prevent and treat allergic responses, and to develop anti-arthropod vaccines (Andrade *et al.*, 2005).

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The phenomenon of host immune response against saliva vector has been studied by Donovan *et al*. (2007) who reported that animal models previously exposed to *Anopheles stephensi* enhance their immune response and inhibit the development of the parasite in the liver and blood. Their immunity is related to a Th1 immune response, with significant production of interferon (IFN)-γ, interleukin (IL)-2 and IL-12. In respond to this problem, blood-feeding arthropods have evolved salivary immunomodulatory factors which prevent host from becoming sensitized to the saliva or even retard deleterious host responses. Such factors induce a Th2 deviation of host's immune response, which favors insect survivor (Andrade *et al*., 2005). Scheneider *et al* (2004) reported the opposite results on previous findings of Donovan *et al*. (2007). Salivary gland extract of *Ae. aegypti* were co-inoculated with Sindbis virus into mice would increase the immune response toward the Th2 (IL4 and in IL-10 cytokines were increase), whereas the IFNχ and IFNβ were significantly decreased. Similar results were reported by Schneider & Higgs (2008) in which a high concentration of salivary proteins was found to be immmunosuppressive.

In this regard SG proteins of *Ae. aegypti* may modify hemostatic responses and induce both cellular immunity and the production of specific antibodies. Therefore, individual human sera response against protein extracts from salivary gland of *Ae. aegypti* is important in determining the factors increasing the transmission of pathogen to human host.

MATERIALS AND METHODS

Rearing of *Ae. aegypti* and Salivary Gland (SG) Dissection

Mosquitoes larvae were collected and reared under strictly identical standard conditions of 28°C and 60% relative humidity at Zoology Laboratory, Department of Biology, Faculty of Natural Sciences, Jember University. Mosquitoes were supplied with a cotton wool pad soaked in 10% sucrose solution. The salivary glands from adult mosquito females were dissected using a fine entomological needle under a stereomicroscope at 4 magnification. Then the salivary glands were pooled into a microcentrifuge tube on ice in phosphate-buffered saline (PBS) and PMSF, then stored frozen at -20^{o}C until needed.

Salivary Gland Protein Extraction

Salivary glands in PMSF and PBS was added with lysis buffer (1:1). The lysis buffer containing 1.5 mM MgCl2, 10 mM tris HCl, 10 mM NaCl, 1% Nonidet P-40, 2 mM EDTA NaOH. After being homogenized with micropistile, sonicated in water bath for 30', then the mixture was centrifuged at 12.690 rpm for 15' in 4 ^{o}C. Supernatant will be concentrated by using eppimembran and centrifuged at 10.000 rpm in 4 ° C by the repeated the procedure for several times so that the concentration becomes more dense, and protein concentration of these salivary gland extract reached 0.69 ug/ul. Salivary gland proteins were then stored at -20^{o}C until used.

Preparation of Blood Sera DHF Patient and Healthy People

Sera sample from DHF patients were collected from endemic area, while sera from healthy people were collected from both endemic area and non endemic area. All participants were informed for their consent to take part in the study following the protocol ap-

proved by the Ethical Committee, Faculty of Medicine, Brawijaya University and Jember University.

SDS-PAGE & Western Blotting

Total proteins from salivary gland extract were separated by 12% SDS-PAGE. The gels were stained with commassie brilliant blue (CBB) R-25 to visualize the proteins. Proteins were transferred to a PVDF membrane under constant current (100 MA) for 1 hour by using semidry Western Blotting. The membranes were blocked for 1 hour with 5% skimmed milk in TBS. Each membrane strip was incubated with a human sera (1:500) overnight at 4^{o}C. Membranes were then incubated with secondary antibodies of anti-human IgG antibodies (goat) AP-conjugated (1:5000) for 2 hours on shaker. Color development was done with NBT-BCIP phosphatase substrate. Prestained broad range molecular weight markers of 7-250 kDa (Intron cat 24084, 24085) were used for estimating protein size.

RESULTS AND DISCUSSION

Salivary Gland of female *Ae. aegypti*

The salivary glands of adult female *Ae. aegypti* have a distinctive tri-lobed structure consisting of a single medial and two lateral lobes (Figure 1). The lateral lobes were divided into two regions, the proximal and distal. The structure of *Ae. aegypti* salivary gland is in pairs, and connected by salivary duct (Juhn *el al*., 2011). Their salivary glands produce proteins containing a number of pharmacologically active components that counteract vertebrate hemostasis, and thus allowing the mosquito to feed successfully such as vasodilator, anti-clothing, and anti-hemostatic protein. It plays a role in pathogen transmission and may induce an immune response in vertebrate host (Valenzuela *et al*., 2002, Cornelie *et al*., 2007, Waitayakul *et al*., 2006).

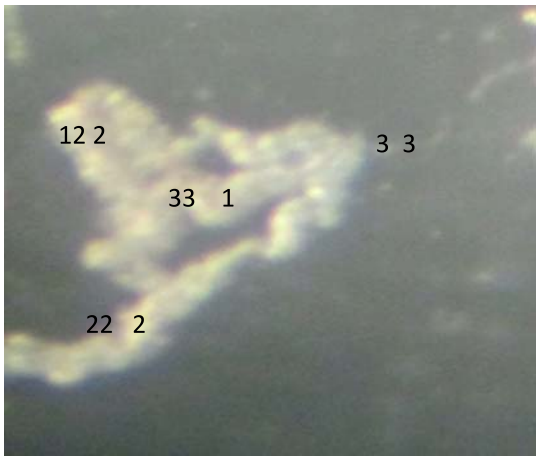

Figure 1. Single salivary gland dissected from a female *Ae. Aegypti.* The salivary gland is comprised of single medial lobe (1) and two lateral lobes (2), the salivary duct connects all salivary gland lobes (3).

Protein profile of SGE *Ae. aegypti*

Identification of protein profile from SGE of *Ae. aegypti* by using SDS-PAGE showed the occurrence of at least 15 bands with different molecular weight ranging from 15 to 255

kDa. Seven dominant bands were detected i.e, ~255, 56, 42, 31, 27, 26 and 15 kDa (Figure 2). Previous study by Wongkamchai *et al* (2010) reported that 13 proteins bands were detected from salivary gland of *Ae. aegypti* with molecular weight ranging from 33.5 to >88.5 kDa. Meanwhile, Machain-Williams *et al*., (2012) reported seven prominent bands with approximate molecular masses of 68, 46, 36, 30, 19, 17, and 14 kDa using by non denaturing PAGE.

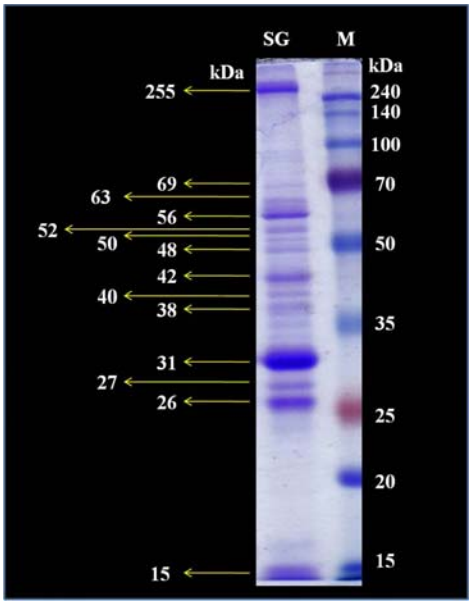

Figure 2. Protein profile of SGE from *Ae. aegypti* form isolation of 100 pairs SG. (SG) Salivary Gland, (M) marker

Immunogenic Proteins of SGE *Ae. aegypti*

Cross reaction test of human sera from healthy people in endemic and non-endemic area, and dengue patients againts SGE of *Ae. aegypti* to distinguish the response and to identify the immunogenic proteins was done by Western Blot Analysis. Two immunogenic proteins (bands) were able to cross-react with sera samples from healthy people and DHF patient in endemic area (protein of 31 kDa and 56 kDa). Sera samples of healthy people from non endemic area were collected from individuals living in subtropical country who have never traveling to tropical countries. They did not show an immunogenic reaction with SGE of *Ae. aegypti*, and their sera were not able to cross-react with the SG protein extract (Figure 3).This result indicated that people living in endemic areas have specific proteins recognized by antibodies of person who frequently exposed by *Ae. aegypti* saliva. These proteins were not found in healthy people from non-endemic areas. Similiar result was reported by Pradines *et al*. (2007) in which the development of antibody response against *An. gambiae* and *Ae. aegypti* saliva increased significantly in travelers who transiently exposed to vector bites in tropical area. Cornelie *et al*., (2007) reported that the development of natural antibodies response for people living in endemic area were due to frequent exposesure to vectors' saliva. Children in malaria endemic areas had developed a spesific IgG response against several proteins of *An. gambiae* saliva.

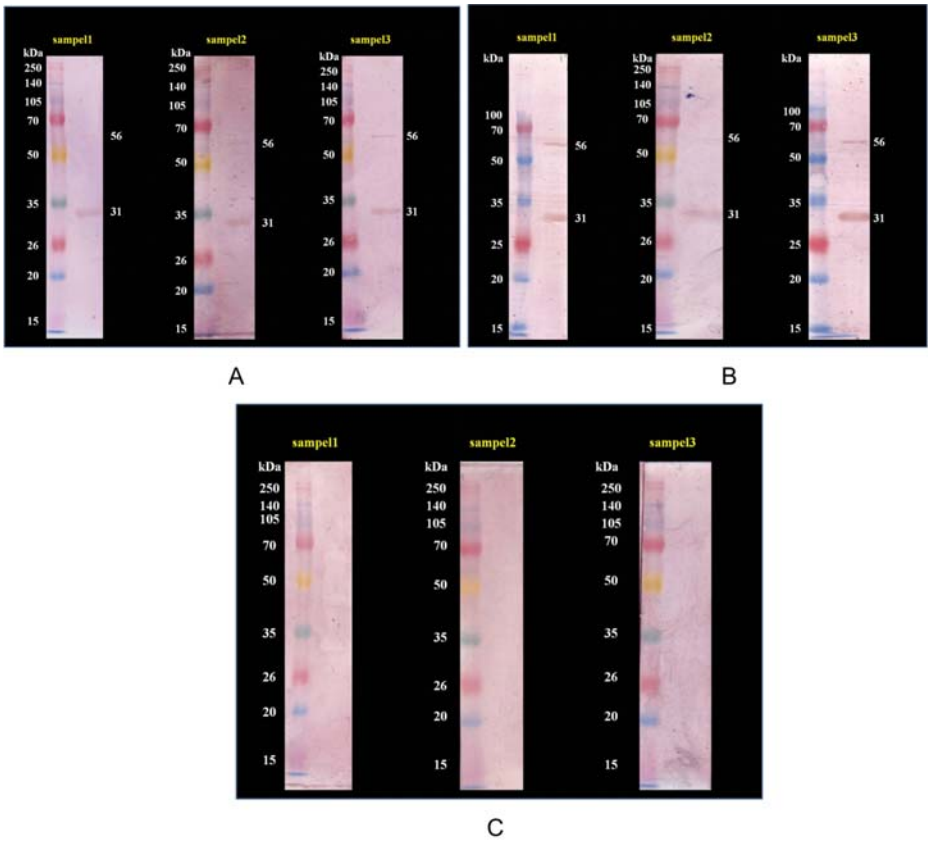

Figure 3. Two immunogenic proteins of SGE from *Ae. aegypti* were identified: 56 and 31 kDa in sera sample from healthy people and DHF patient in endemic area.
(A) healthy people from endemic area , (B) DHF patient (C) healthy people from non endemic area

CONCLUSION

1. Protein profiles of SGE from *Ae. aegypti* showed that 15 bands were identified, with different molecular weight ranging from 15 kda up to 255 kda. Seven dominant bands were detected i.e ~255, 56, 42, 31, 27, 26 and 15 kDa.
2. Two immunogenic proteins of SGE *Ae. aegypti* were detected, the ~31 and 56 kDa. These proteins were able to cross-react with sera samples from people who were previously exposed to mosquitoes bites, and not in people who had not been exposed.

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