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TOPIC 1: MOLECULAR BIOLOGY, GENETIC AND BIOINFORMATIC

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, thus, influence transmission of its pathogens from arthropods vector to human host. *Ae. aegypti* is the main vector for transmission of Dengue viruses into human. The objective of this research is therefore to elaborate individual human sera response against protein extracts from salivary gland of *Ae. aegypti* that mediate the infection of Dengue Viruses. We crossed react human sera from healthy people in endemic and non-endemic area, and dengue patients againts SGE of *Ae. aegypti* to distinguish to identify the immunogenic proteins by Western Blot Analysis. About 15 protein bands of SGE from *Ae. aegypti* ranging from 15 kDa up to 255 kDa were identified after 12% SDS-PAGE. 7 dominant bands were detected i.e ~255, 56, 42, 31, 27, 26 and 15 kDa. Two immunogenic proteins (bands) i.e. ~31 and 56 kDa were appeared only in samples from humans who were previously exposed to mosquitoes bites, and not in humans who had not been exposed. Therefore, these immunogenic salivary proteins may serve as indicators for the immune response in humans against protein from salivary gland *Aedes aegypti*.

Key words: Immunogenic proteins, Salivary Gland, *Aedes aegypti*,

Introduction

Dengue virus (DV) causes dengue fever and the more severe conditions 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 the mosquito vectors *Aedes aegypti* as a main vector and *Aedes albopictus* as a secondary vector. The vectors acquire the pathogens by feeding on infected hosts and then transmit them by regurgitation during a subsequent blood meal (Ader *et al*., 2004). Blood feeding required for nutrition, egg development and survival (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 are also antigenic and immunogenic, 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) and 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 saliva (Cornelie *et al*., (2007). That 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).

The phenomenom of host immune response against saliva vector, can be shown by some studies such as, Donovan *et al,*. (2007), reported that animal models previously exposed to *Anopheles stephensi* can 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. To face 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), has reported the opposite results of that Donovan's research. 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 IFNɣ and IFNβ significantly decreased. The similar results were also reported by Schneider & Higgs (2008), a high concentration of salivary proteins were immmunosuppressive.

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

Materials and Methods

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

Mosquitoes larvae were collected and reared under strictly identical standard conditions 28°C and 60% relative humidity at Zoology Laboratory of Biologi Department-Natural Science Jember University. Mosquitoes are 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.

2. Salivary Gland Protein Extraction

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

3. Preparation of Blood Sera DHF Pasient and Healthy People

Sera sample from DHF patients were collected in endemic area and healthy were collected in endemic area as well as non endemic area. All participant gave their informed consent to take part in the study and the protocol was approved by the Ethical Committee of Medical Faculty, University of Brawijaya and Jember University.

4. SDS-PAGE & Western Blotting

Total protein from salivary gland extract were separated by 12% SDS-PAGE. 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 using by 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 anti-human IgG antibodies (goat) AP-conjugated (1:5000) for 2 hours on shacker. Color development was done with NBT-BCIP Phosphatase substrate. Prestained broad range molecular weight markers (7-250 kDa) (Intron cat 24084, 24085) were used for estimating protein sizes.

Results and Discussion

1. 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, lateral lobes (figure 1). Lateral lobes defined in two regions proximal and distal lateral . *Ae. aegypti* salivary gland is pairs in structure, and connected by salivary duct. (Juhn *et al*., 2011). Their salivary glands produce proteins contain a number of pharmacologically active components that counteract vertebrate hemostasis, thus allowing the mosquito to feed successfully such as vasodilator, anticlothing, and antihemostatic protein. It plays a role in pathogen transmission and can induce an immune response in the vertebrate host (Valenzuela *et al*., 2002, Cornelie *et al*., 2007, Waitayakul *et al*., 2006).

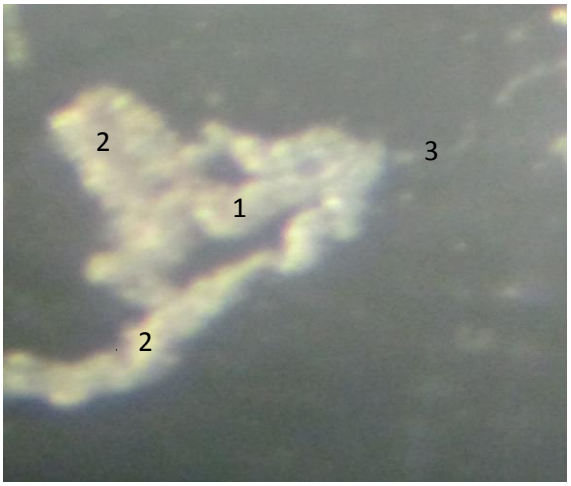

Figure 1. Single salivary gland dissected from a female *Ae. aegypti,* the salivary gland is comprised single medial lobe (1). two lateral lobes (2), the salivary duct connects all salivary gland lobes (3).

2. Protein profile of SGE *Ae. aegypti*

Identification of the protein profile from SGE of *Ae. aegypti* by using SDS PAGE, many protein bands were identified at least 15 bands with different molecular weight ranging from 15 kDa up to 255 kDa. About 7 dominant bands were detected i.e ~255, 56, 42, 31, 27, 26 and 15 kDa (Figure 2). Previous study reported by Wongkamchai *et al* (2010), salivary gland proteins of *Ae. aegypti* were detected 13 bands with a molecular weight ranging from 33.5 to >88.5 kDa. Whereas seven prominent bands were identified in salivary protein, with approximate molecular masses of 68, 46, 36, 30, 19, 17, and 14 kDa using by non denaturing PAGE (Machain-Williams *et al*., 2012).

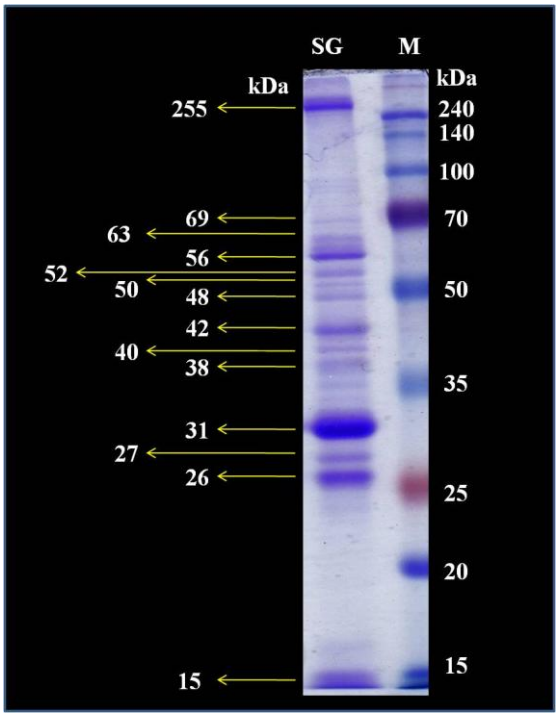

Figure 2. Protein profile of SGE *Ae. aegypti* isolation of 100 pairs SG. (SG) Salivary Gland, (M) marker

3. Immunogenic Proteins of SGE *Ae. aegypti*

Crossed react human sera from healthy people in endemic and non-endemic area, and dengue patients againts SGE of *Ae. aegypti* to distinguish to identify the immunogenic proteins by Western Blot Analysis. Two immunogenic proteins (bands) were able to cross-react with sera sample from healthy people and DHF patient in endemic area (protein of 31 kDa and 56 kDa). Sera sample of healthy from non endemic area individuals living in sub tropical country who have never traveling to tropical countries. They did not show an immunogenic reaction with SGE *Ae. Aegypti*, their sera were not able to cross reacted with the SG protein extract (figure 3).This results indicates that people living in endemic areas have specific proteins were recognized by antibodies of person who frequent exposed by

saliva, since these proteins are not found in healthy non-endemic areas. The similiar result was supported by research of Pradines *et al*.,(2007), the development of antibody response against *An. Gambiae* and *Ae. aegypti* saliva increased significantly in travelers transiently exposed to vector bites in tropical area. Cornelie *et al*., (2007), reported that the development of natural antibodies response in the people living in endemic area due to frequent exposed by saliva. The children in the malaria endemic area had developed a spesific IgG response against several proteins of *An. Gambiae* saliva.

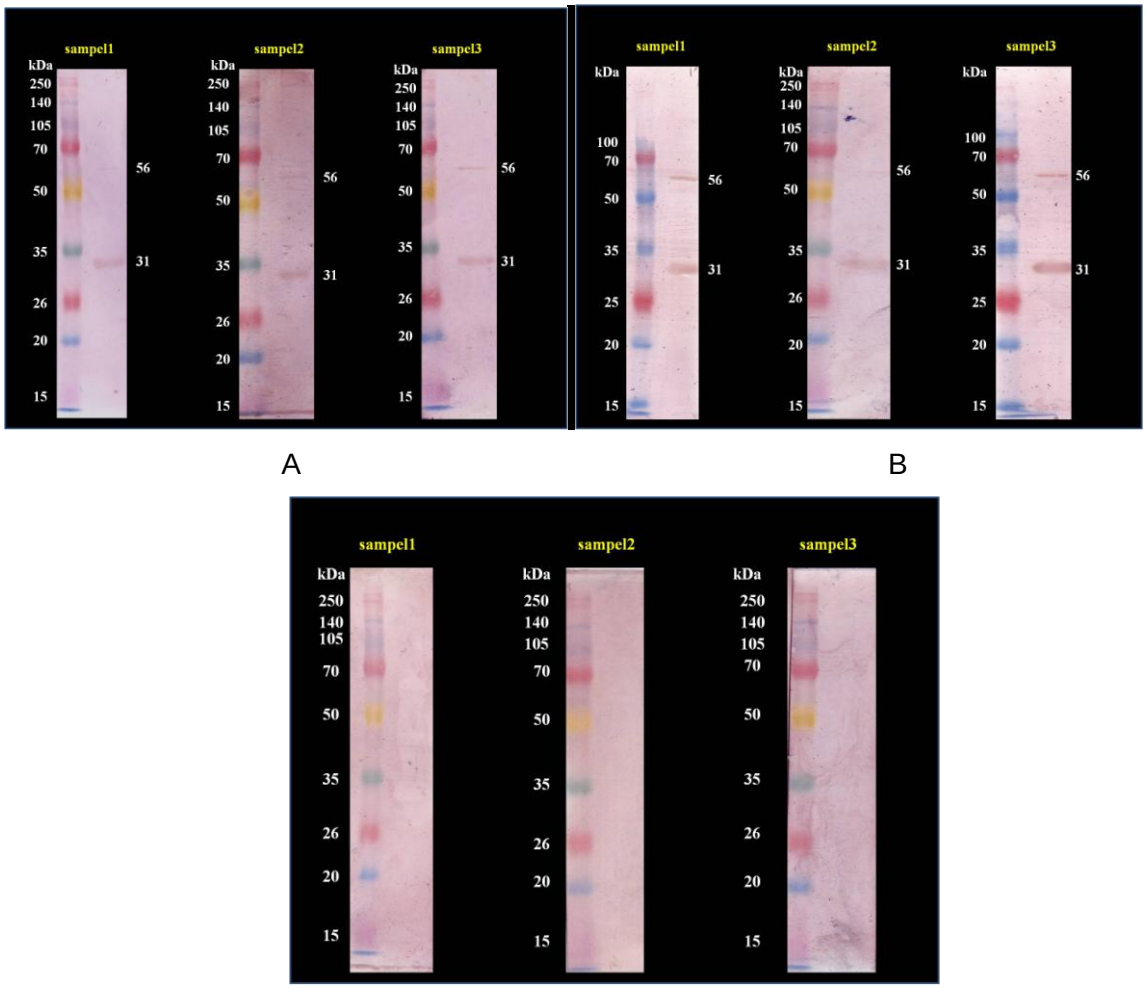

Figure 3. Two immunogenic proteins of SGE *Ae. aegypti* were identified i.e 56 and 31 kDa in sera sample from healthy people and DHF patient in endemic area. (A) healthy people from endemic area , (B) DHF patient (C) healthy people from non endemic area

CONCLUSION

1. The protein profiles of SGE *Ae. aegypti* were identified 15 bands with different molecular weight ranging from 15 kda up to 255 kda and 7 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 ~31 and 56 kDa, able to cross-react with sera sample from humans who were previously exposed to mosquitoes bites, and not in humans who had not been exposed

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