REVIEW ARTICLE

Human Antibody Response to Dengue Vector Salivary Proteins: A Mini-Review

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ABSTRACT

The widespread expansion of dengue is alarming. The challenges of vector control strategies warrants the implementation of alternative assessments to curb its invasion. This review summarizes the contemporary knowledge on the credible use of human antibody response towards mosquito salivary protein as predictive markers for dengue infection. The literature was sourced from electronic databases such as Scopus, PubMed, Springer Link, Wiley Online Library and Science Direct. Keywords such as "salivary proteins", "biomarker", "dengue" "antibody response" were utilised. The evaluation of antibody reaction towards salivary peptides in mosquitoes is a practical immuno-epidemiological method that could be further explored to heighten the diagnostic monitoring of dengue transmission.
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INTRODUCTION

Aedes mosquitoes are significant vectors that are frequently associated with the persistent emergence and re-emergence of arboviral diseases of clinical significance. The global incidence and dramatic resurgence of dengue in recent years have caused major public health concerns. 400 million dengue cases were reported with 100 million people experiencing sickness and 22,000 deaths (1). According to the World Health Organization, 420,453 cases and 1,565 deaths associated with dengue were reported in the Philippines in 2019. Singapore documented a total number of 15,622 dengue cases in 2019 which was higher compared to 2018 (2). Similarly, 50,511 positive cases and 88 deaths related to dengue were reported in Malaysia in the first six months of 2020 (3).

Many strategies have been implemented to control the population of mosquito-borne viral vectors including the worldwide implementation of integrated vector management systems (2). Entomological approaches for vector control including the utilization of insecticides, repellents, breeding sites, aspirators, human landing catches have had significant impacts over the years but have not eliminated the vectors. In addition, such methods may have drawbacks in terms of scale measurement and could be deemed unsuitable for either individual detection or point-of-care testing (4-5); warranting the exploration of alternative measures to complement existing ones. To date, there is no commercially available vaccine or any therapeutic agent to combat dengue, hence the implementation of vector control programs remains the best practice to control the vector population and to reduce the risk of dengue transmission (6).

Pathogen detection and entomological indices are means that can be used to assess human exposure to mosquito bites (7). Currently, antigen detection methods using Rapid Diagnostic test (RDT) and dengue non-structural protein 1 (NS1) antigen test remain the most rapid and accurate diagnostic tools. Existing serological and molecular-based methods such as Enzyme-Linked Immunosorbent Assay (ELISA) and Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) are also widely used to detect dengue through the detection of Immunoglobulin (Ig) M and IgG anti-dengue antibodies (2). In addition, the analysis of human antibody response towards dengue vector salivary protein could help measure the intensity of vector exposure and can act as a marker in evaluating dengue transmission risk (8). This is because the host blood is essential to

vectors and mosquito salivary protein is vital during the blood-feeding process and for dengue virus (DENV) transmission (6).

Mosquito saliva comprises many compelling immunogens that could stimulate the production of antibodies in humans (9). The level of antibody produced can be correlated with the strength of exposure to vector bites (10). Previous studies have shown that IgE and IgG responses towards *Aedes aegypti* saliva increased during rainy seasons (11) and among individuals residing in urban areas where *Aedes aegypti* is found (5). Such findings signify that Aedes saliva has a substantial influence on host immune response, thus justifying the rationale of it being utilized as a biomarker to aid laboratory diagnosis. This review aims to explore the association of human antibody response towards *Aedes aegypti* and *Aedes albopictus* salivary proteins and its potential usage as a biomarker. The correlation of human immune response to mosquito bites with dengue severity will be discussed.

METHOD

An electronic search without time restrictions was performed to conduct a literature search using the following databases: Scopus, PubMed, Springer Link, Wiley Online Library and Science Direct. The following terms were used in the search strategies: salivary proteins", "biomarker", "dengue" "antibody response". Reviews, original articles, short communications were screened. Manual searches through the references of selected full texts were performed to retrieve relevant literature. A total of 30 articles were included in this study. Only peer-reviewed articles published in English, with full text available were selected.

RESULTS AND DISCUSSION

Salivary biomarkers of mosquito bites

The human immunological response towards mosquito saliva is both imperative and multifaceted involving three main processes namely haemostasis, inflammation, and immunity in general. Homeostasis is a natural mechanism to stop or prevent bleeding that involves blood coagulation and vasoconstriction while inflammation is a response triggered by damage to living tissues. Importantly, immunity is related to a host immune response towards the exposure of antigen or pathogen during the host-vector contact (12). In this context, both the innate and adaptive responses work simultaneously and in conjunction to counteract the foreign salivary protein of Aedes mosquitoes (13).

Vertebrate host blood provides complete nutrition, egg development, and maturation that are essential for the survival of Aedes species (14). Mosquito bites during the blood-feeding process are pivotal in ensuring sufficient interaction between the Aedes vector and humans. The saliva in mosquitoes contains an intricate concoction of salivary proteins and components with multiple functions to counter-attack the host defence mechanism in inhibiting the blood-feeding process. Specifically, it contains vasodilatory and immunomodulatory factors that catalyse blood-sucking and potentially aids the inhibition of homeostasis and vasoconstriction (12-13).

The immunomodulators have profound effects on the human immune system which enables the manifestations of symptoms related to allergic response such as skin itchiness and redness. Arthropod saliva is also capable of inducing an antibody response in individuals exposed to the salivary protein due to its antigenicity and immunogenicity. It was recently demonstrated that mosquito salivary proteins of *Aedes aegypti* can induce immunological reactions that echo the depth of human vector contact. The development of IgG antibodies towards *Aedes aegypti* salivary gland extracts (SGE) was found to be expressively greater among participants residing in houses that contains domestic containers that support the growth of Aedes larvae (8). This suggests that saliva from Aedes mosquitoes contain active protein components that are responsible for the production of specific antibodies in humans. Additionally, a study using humanized mice engrafted with human hematopoietic stem cells demonstrated that mosquito bites are capable of altering cytokines levels, mainly increasing the anti-inflammatory and T helper (Th) 2 cytokines at 7 days post-bite (13).

Assessment of the human contact to arthropod vector bite can be made feasible by determining the human-vector contact. This was previously demonstrated by measuring the intensity of vector exposure in relation to the antibody reaction towards vector salivary protein (15). Previously, human antibody responses to the saliva of Anopheles gambiae, the vectors for malaria (11), and Triatoma vectors for Chagas disease (16) have successfully shown to be a well-founded immunologic marker for vector exposure. Hence, the development of a sensitive and specific diagnostic tool to assess human exposure to Aedes vector bites by utilizing vector salivary biomarkers would be of diagnostic value. Several studies in the past have tested the efficacy use of such an approach with promising findings.

In previous studies, protein bands from 14 kDa to 68kDa (17) and 13 protein bands from 26 kDa to 255 kDa (12) were detected in the saliva of *Aedes aegypti* extract by polyacrylamide gel electrophoresis. Two dominant bands at 31 kDa and 56 kDa were found to be specifically induced as a result of immunological response in individuals living in endemic areas. In addition, five secreted proteins including are likely to be involved in the blood-feeding process (14). The D7 proteins (37-kDa) in particular are likely to stimulate an antibody-mediated immunological response due to the

frequent contact with *Aedes aegypti* saliva particularly in individuals living in dengue-endemic areas and has been associated with inducing allergic reactions in infected individuals (18). Furthermore, several known salivary factors has been identified in *Aedes aegypti* with known or suspected roles in host immunity. This includes the correlation of the saliva with its role in enhancing virus transmission and its effects on host susceptibility and disease progression, as summarised by Guerrero et al., 2020 (19) in Table I.

Table I : Summary of salivary factors in *Aedes aegypti* and their known or suspected role in host immunity

Salivary factors	Roles in host immunity
A. aegypti venom allergen-1 (AaVA-1)	Enhances viral replication
Neutrophil stimulating factor 1 (NeSt1)	
Anticlotting serpin-like protein (AT)	
Adenosine deaminase (AD)	
Putative 34 kDa family secreted salivary protein	
Putative secreted protein (VA)	
LTRIN	Decreases the expression of pro-inflammatory cytokines
Serine protease CLIPA3	Enhances infection
A. aegypti bacteria-responsive protein 1 (AgBR1)	
miRNA-100	Possible effect on the regulation of immune cell activity and influences viral replication
miRNA-125	

Human antibody response to dengue vector salivary protein

The analysis of human vector contact is crucial to measure the transmission probability of arthropod-borne disease. In particular, the evaluation of human antibody response towards mosquito salivary proteins administered by hematophagous arthropods during the blood-feeding process could be a potential immuno-epidemiological marker that can be utilised in vector control programs. For this reason, the human antibody responses to the saliva of various vectors, including Phlebotomus (Leishmaniasis) (20) and Triatoma (Chagas disease) (16) Nascimento, 2001) have been used in the past as a credible biomarker for detection. The quantification of specific antibody response to Aedes salivary protein enables direct estimation of exposure to vector bites. In particular, its quantitative utilisation in methods such as ELISA could be a useful diagnostic tool and a measure of human exposure to mosquito bites (8).

The capacity of mosquito saliva to regulate the host immune response has been predominantly studied in *Aedes aegypti* (19). Nevertheless, there have been studies that focused on other vectors too such as *Aedes albopictus*, Aedes polynesiensis and Anopheles gambiae. Studies that target human exposure to Aedes saliva mainly focus on the environmental parameters to determine the density and fluctuation of Aedes vectors. It has been demonstrated that rainfall density and geographical phenomena can intensely impact the vector density and population distribution (4, 21-22). As such, the evaluation of human contact with vector saliva during the wet season is more advantageous in comparison to other seasons. A study focusing on Senegalese children in rural areas of Africa demonstrated that both levels of specific IgE and IgG4 antibody reactions to the mosquito saliva of *Aedes aegypti* increased during the rainy season. It was documented that 56.25% of the responders developed specific IgG4 responses against *Aedes aegypti* saliva in December, in contrast to only 47.2% of the responder in July. This could be due to the high proliferation and propagation of Aedes vectors during the rainy season with less risk of human contact with Aedes bites (4).

However, vector exposure intensity in a population could vary between individuals. 88% of the exposed individuals in Reunion Island developed specific IgG responses against *Aedes albopictus* SGE that was remarkably dissimilar compared to unexposed individuals. Anti-SGE IgG responses developed in immune responders may be a valid and dependable biomarker to evaluate human exposure to vector bites. Nevertheless, epidemiological factors such as the timing of contact, genetic background, and immunological tolerance could be confounding factors resulting in various immune responsiveness (5). Additionally, the dependable use of human antibody response as an indicator of the contact to mosquito bites depends on the specificity of these biomarkers. This in turn necessitates testing for the likelihood of cross-reactive epitopes between different mosquito salivary proteins in *Aedes aegypti* and *Aedes albopictus*. Data on serological screening of the detection and evaluation of IgG response against mosquito saliva in Tahiti and Moorea islands revealed high IgG sensitization (97.9% and 68.1% respectively) of the resident island community against *Aedes aegypti* and Aedes polynesiensis saliva (15).

These findings highlight the fact that the antibodies produced upon contact were highly heterogeneous, as observed in other studies utilizing similar types of Aedes SGEs in Reunion Island (5) Columbia (23) and Bolivia (24). The study also screened the sera of individuals against SGEs of both *Aedes aegypti* and *Aedes albopictus* in four different localities where Aedes polynesiensis was absent to screen for the risk of cross-reaction. Data analysis from three *Aedes aegypti* exposed areas (Martinique, New-Caledonia, Bolivia) revealed no cross-reactions with Aedes polynesiensis SGEs. Similar results of no clear cross-reactivity between *Aedes aegypti*, *Aedes albopictus* and Aedes polynesiensis

SGEs from sera of individuals in the Reunion Islands were also obtained. These findings postulates that IgG antibody responses are species-specific and expression of an immunogenic protein in the sialome of the Aedes genus differs according to various species (25).

Proteomics, transcriptomic and metabolic studies conducted in recent years have exposed the intricacy and convoluted nature of the mosquito salivary components and have enabled the identification of genus-specific mosquito salivary proteins (14-15). The determination of such proteins is favourable and pertinent when developing a sensitive and specific immunological assay that could be targeted against a wide variety of mosquito vectors (7). Anopheles gambiae gSG6 or the gsg6-p1 peptide has been used to evaluate IgG responses to anopheline Afrotropical malaria vectors in African countries (26-27). Previous studies have provided insights with regards to the immunogenic properties of *Aedes albopictus* 34k2 salivary protein (al34k2) that can elicit an immune response that is culicine-specific. A study showed that higher anti- al34k2 IgG response is apparent shortly after the summer exposure and declined during non-exposure to *Aedes albopictus* bites during the winter seasons (7).

Another longitudinal study on the IgG response targeting the specific and antigenic salivary protein of *Aedes aegypti*, N-term-34kDa peptide suggests a newly paved way to assess human exposure to these Aedes bites. The human IgG responses to the N-term-34kDa salivary peptide of *Aedes aegypti* were quantified in individuals, pre and post vector control implementation in La Reunion Island. Initial observation demonstrated that 88.23% of individuals exposed to Aedes bites presented a high IgG response to Aedes N-term-34kDa salivary protein compared to those with unexposed individuals. Results have shown that the median of the IgG responses among the exposed La Reunion Island residents was 3 folds higher than the cut-off value, supporting the findings of the study (28).

Dengue transmission risk prior exposure to Aedes vector bites

It has been demonstrated that the inoculation of mosquito salivary protein during infection strongly correlates with enhanced pathogenesis of arbovirus disease (29). Such initiation can greatly facilitate the transmission of the pathogen inside the host system (30-31). The precise correlation of human immune reactions to mosquito salivary protein with dengue intensity, however, remains unclear. A retrospective study suggested that individuals previously exposed to mosquito salivary protein might display a vital role in the outcome of dengue infection due to the probable effects of previous exposure to arthropod salivary proteins. Higher antibody titers to mosquito salivary protein were found in individuals infected with DENV2 compared to non-infected patients (17). Individuals with greater exposure to arthropod vectors are at higher risk of getting infected (32) due to longer human vector contact during a blood meal. In addition, the existence of more than two serotypes in a localized area increases the chances of developing a more critical dengue infection.

A study by Cardenas et al., (9) demonstrated that 64% of the sample population in Los Patios and Ocaca, in the northeast of Colombia presented DENV non-structural protein NS1-IgG implying a greater risk of secondary infection. This correlates with the detection of NS1-IgG antibodies after 14 days in a primary infection (33). In this context, early detection of secondary infection is an important factor that can facilitate a better prognosis. Thus, this finding could positively suggest the utilization of human antibody response against mosquito salivary protein as a relevant immunoassay to measure vector exposure and risk of disease transmission.

CONCLUSION

Dengue vector surveillance relies predominantly on preventive and vector control implementation measures. The global incidence of dengue necessitates direct estimation of dengue transmission risk, which may include the evaluation of antibody response in relation to human-vector contact to synergistically augment existing classical entomological methodologies. Individuals exposed to mosquito saliva tend to develop antibodies in response to the foreign salivary protein injected into the human host during the blood-feeding process. Due to the immunogenic properties of mosquito salivary protein, individual exposure to mosquito bites with primary and secondary dengue infection may reveal an interrelation between the history of infection and the level of exposure to vector bites. The intensity of the bites and the risk of viral transmission could be used to develop new immunological assays. As such, the use of species-specific salivary antibodies or peptides in Aedes mosquitoes is promising and could be utilized as a dependable marker to further detect spatial or temporal variations of human exposure to Aedes bites.

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