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Antigenicity of envelop and non-structural proteins of dengue serotypes and their potentiality to elicit specific antibody

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PEER REVIEW

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ABSTRACT

Objective: To find out the antigenic nature of envelop (E) and non-structural (NS) proteins and their ability to induce specific antibodies, and to investigate specific antibody produced by specific dengue virus (DENV) serotypes.
Methods: Amino acid sequences of E and NS proteins of dengue serotypes were analysed by using VaxiJen antigen predicition server. The transmembrane of topology analyses were conducted by using transmembrane prediction using hidden markov models. The Hex dock server was used for docking.
Results: The antigenicity score and exomembrane potentiality of E and NS proteins were calculated. All those proteins were antigenic; these antigens were made to interact with antibodies such as immunoglobulin A, immunoglobulin G and immunoglobulin M. Higher energy values of immunoglobulin M were found in DENV-1 and DENV-2, and more energy values were found in immunoglobulin G of DENV-3, DENV-4, NS-1, NS-3 and NS-5.
Conclusions: In the present study, DENV-1 and DENV-2 are positive to immunoglobulin M and involved in the primary infection. DENV 3, DENV 4 and all the NS proteins (NS-1, NS-3, NS-5) which elicit immunoglobulin G are involved in the secondary infection.

1. Introduction

Dengue is a mosquito-borne viral infection found in tropical and subtropical regions worldwide. The World Health Organization estimates that today more than 2.5 billion people are at high risk of dengue disease[1]. Currently, the disease is endemic in more than 100 countries, and is spreading throughout the world. Dengue disease is caused by four antigenically related distinct serotypes, namely, dengue virus (DENV)-1, DENV-2, DENV-3 and DENV-4 in which only DENV-2 and DENV-3 are mostly found in tropical countries[2,3]. The distinctions among the serotypes are based on their antigenicity and immunogenic property in the human body.

The E protein is presented on the viral membrane which is a functional protein molecule that binds to receptors on the host cell membrane; it is also a major antigen against host protective immunity which induces neutralizing antibody[4-6]. During viral infection, the adsorption of viral particles is initiated by binding of E protein to receptor molecules which is presented on the host cell membrane. Subsequently, the adsorbed viruses are taken into the cell by endocytosis. DENV-1, DENV-2, DENV-3 and DENV-4 are known to cause dengue fever[6]. The dengue virions are spherical and icosahedral in shape with a positive-sense RNA genome that has a single open reading frame, codes for three structural proteins [capsid, membrane and envelop (E) proteins] and forms the components of the virion and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) which are involved in the replication cycle of the virus[7,8]. The E protein is responsible for host cell receptor recognition and the induction of neutralizing antibodies from the host. Therefore, E protein has been an attractive candidate for its use in the development of an effective and safe recombinant vaccine[9,10]. E protein plays an important role during the life cycle of the DENV, including cell receptor binding and entry into the host cell via membrane fusion.

2. Materials and methods

Docking studies were performed for E and NS proteins of antigens by using Hex 6.3 module of Schrodinger Suite.

2.1. Computational methods with Hex version 6.3

All computational studies were carried out by using Hex version 6.3, installed in a single machine running on Intel Core i7 Duo Processor with 1GB radom access memory and 275 GB hard disk

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with black Dell Inspiron version 7.0 as the operating system.

2.2. Antigen prediction with VaxiJen

Amino acid sequences of E and NS proteins of dengue serotypes DENV-1, DENV-2, DENV-3, DENV-4, NS-1 and NS-3 were retrieved in SWISS-Prot database (http://us.expasy.org/sprot)[11] and analyzed by using VaxiJen V. 2.0 antigen prediction server (www.Ddg-pharmofac.netvaxigen)[12].

2.3. Transmembrane of topology with transmembrane prediction using hidden markov models (TMHMM)

Each amino acid sequence was then subjected to transmembrane topology analysis by using TMHMM V. 2.0 prediction server in order to identify exomembrane (surface exposed) amino acid sequences of each protein[13].

3. Results

3.1. Potentiality of E and NS proteins as antigens

Four E proteins of DENV serotypes, causing dengue fever, were selected for the present study. Full length of E proteins was ranging from 480 to 495 amino acids. The antigenicity of the entire E proteins was determined based on the VaxiJen score (Figure1) and topology of the transmembrane (Figure 2). E proteins of DENV having antigenic score > 0.4 were predicted as antigenic. From the results obtained, it was found that all the four E proteins were antigenic (Table 1) and the VaxiJen scores were ranging from 0.6723 to 0.7459 (Table 1). Out of four E proteins, DENV-1 showed the highest VaxiJen score (0.7459). The basic criterion of a good antigen is that it must be exposed outside the membrane. The results of the transmembrane topology analyses revealed that all the four E proteins enzymes were fully exposed outside the membrane, and thereby qualified as antigenic. Thus, the four E proteins showed antigenic property by having VaxiJen score greater than the threshold value (0.4) and the exomembrane topology (Table 1).

VaxiJen results

Model selected: virus

Threshold for this model: 0.4

Your sequence:

MRCVGIGSRDFVEGLSGATWVDVVLEHGSCV
TTMAKDKPTLDIELLKTEVTNPAVLRKLCIE
AKISNTTTDSRCPTQGEATLVEEQDANFVCR
RTFVDRGWGNGCGLFGKGSLLTCAKFKCVTK
LEGKIVQYENLKYSVIVTVHTGDQHQVGNES
TEHGTTATTTPQAPTTEIQLTDYGALTLDCS
PRTGLDFNEMVLLTMKEKSWLVHKQWFLDLP
LPWTSGASTSQETWNRQDLLVTFTAHAKKQ
EVVVLGSQEGAMHTALTGATEIQTSGTTTIF
AGHLKCRLKMDKLTLKGMSYVMCTGSFKLEK
EVAETQHGTVLVQIKYEGTDAPCKIPFSTQD
EKGVTQNGRLITANPIVTDKEKPVNIEAEPP
FGESYIVIGAGEKALKLSWFKKGSSIGKMFE
ATARGARRMAILGDTAWDFGSIGGVFTSVGK
LVHQIFGTAYGVLFSGVSWTMKIGIGVLLTW
LGLNSRSTSLSMTCIVAGLVTLYLGVMVQA

Overall predicition for the antigen = 0.7459 (probable **ANTIGEN**)

Figure 1. Antigenicity of E proteins of DENV-1.

```
# Length:  495
# Number of predicted TMHs:   2
# Exp number of AAs in TMHs: 49.29563
# Exp number, first 60 AAs:    0.00275
# Total prob of N-in:          0.10329
TMHMM2.0      outside      1     442
TMHMM2.0      TMhelix    443     465
TMHMM2.0      inside     466     471
TMHMM2.0      TMhelix    472     494
TMHMM2.0      outside    495     495
```

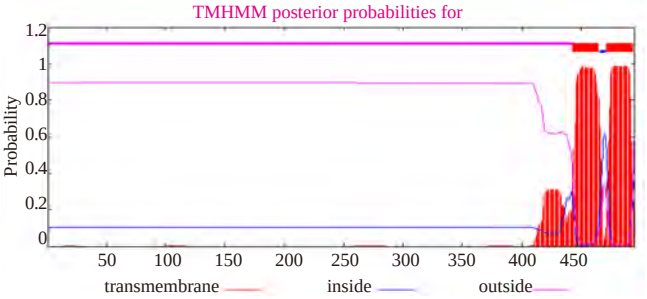

Figure 2. Topology of E proteins of DENV-1.

Table 1

Antigenicity of E and NS proteins of DENV serotypes.

Enzymes	VaxiJen score	Topology	Antigenicity
E protein of DENV-1	0.7459	Exomembrane	Antigen
E protein of DENV-2	0.6749	Exomembrane	Antigen
E protein of DENV-3	0.7395	Exomembrane	Antigen
E protein of DENV-4	0.6723	Exomembrane	Antigen
NS-1 protein of DENV	0.6126	Exomembrane	Antigen
NS-3 protein of DENV	0.4929	Exomembrane	Antigen
NS-5 protein of DENV	0.4600	Exomembrane	Antigen

The important NS proteins, NS1, NS3 and NS5 of DENV serotypes were analyzed for antigenicity. The antigenicity of the entire NS proteins was determined based on the VaxiJen score and topology of the transmembrane. From the results obtained, it was found that all the three NS proteins were antigenic and the VaxiJen scores were ranging from 0.4600 to 0.6126. The results of the transmembrane topology analyses revealed that all the three NS proteins were fully exposed outside the membrane, and thereby qualified as antigenic (Table 1).

3.2. Molecular docking of E and NS proteins with antibodies

The 3D structure of the E and NS proteins were provided with five ligand binding sites except NS-1 in which only two ligand binding sites presented. The number of active binding site was four in the case of DENV-2, DENV-3 and DENV-4. Binding site-2 was recognized as an active binding site for DENV-1 and NS-5. For NS1 and NS3, the active binding site was found in the ligand binding site-1 on the protein molecule. Molecular docking was performed between the E and NS protein antigens of DENV and antibody molecules, immunoglobulin A (IgA), immunoglobulin M (IgM) and immunoglobulin G (IgG) (Figures 3a-3c).

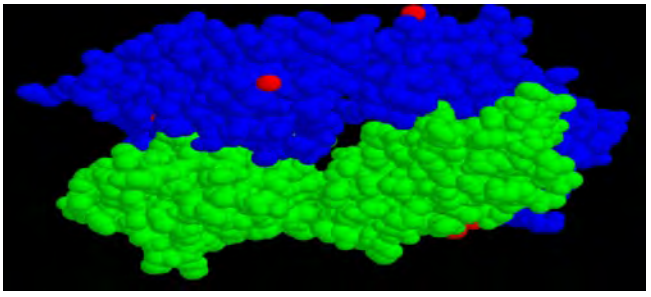

Figure 3a. 3D structure of IgA antibody.

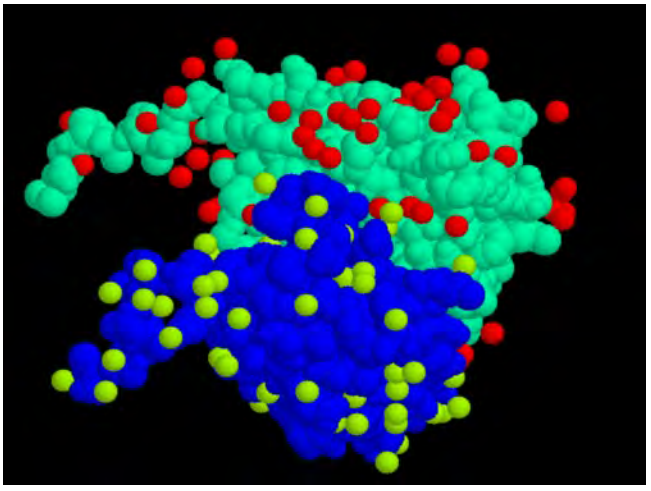

Figure 3b. 3D structure of IgM antibody.

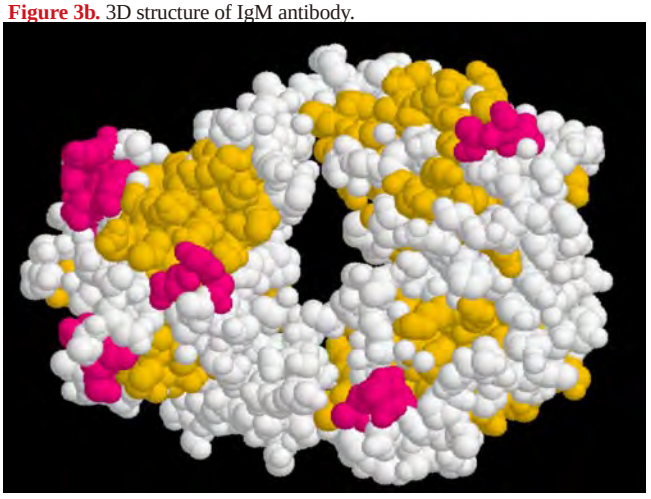

Figure 3c. 3D structure of IgG antibody.

The above target proteins (antigens) and ligands (antibodies) were geometrically optimized. All the ligand molecules were docked against the active sites of the target antigen by using Hex software. The docking results were presented in the form of negative energy values. In the docking studies, higher negative energy values represent high binding affinity between the receptor and ligand molecules, indicating the higher efficiency of the antibodies. Docking of DENV-1 E protein with antibodies IgA, IgM and IgG produced energy values such as -667.20, -689.22 and -666.25 respectively. Among the three antibodies, IgM showed a higher negative energy value of -689.22 than that of IgA and IgG (Figure 4).

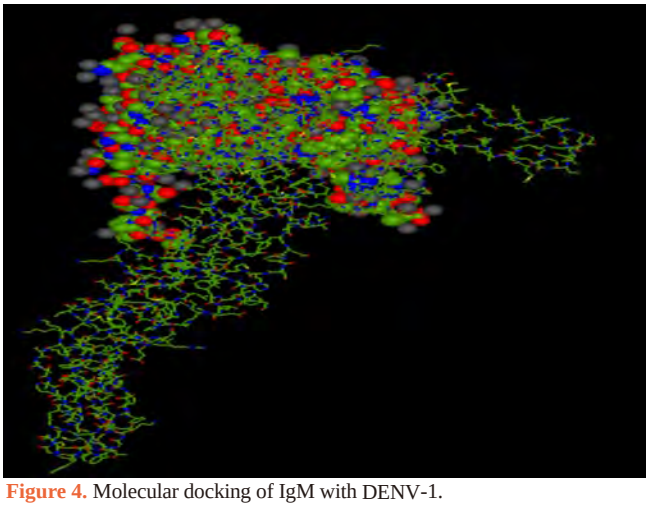

Figure 4. Molecular docking of IgM with DENV-1.

Similarly, all the antibodies were also docked with other three proteins of the DENV serotypes. DENV-2 E protein also showed a higher negative energy value of -650.65 with IgM. When DENV-3 and DENV-4 E proteins were docked with antibodies, IgG showed the higher negative energy values of -681.60 and -699.03 respectively which were higher than that of IgA and IgM (Figure 5). Similarly, NS proteins of the DENV genome were also docked with the three antibodies, IgA, IgM and IgG. When the NS-1 was docked with the above antibodies, IgG showed the higher negative energy value of -778.50 (Figure 6). NS-3 also showed higher negative value of -693.62 when docked with IgG. In the docking between NS-5 and three antibodies IgA, IgM and IgG, IgG showed higher negative value of -730.82. Thus, all the NS proteins showed an effective interaction with the IgG antibody (Table 2).

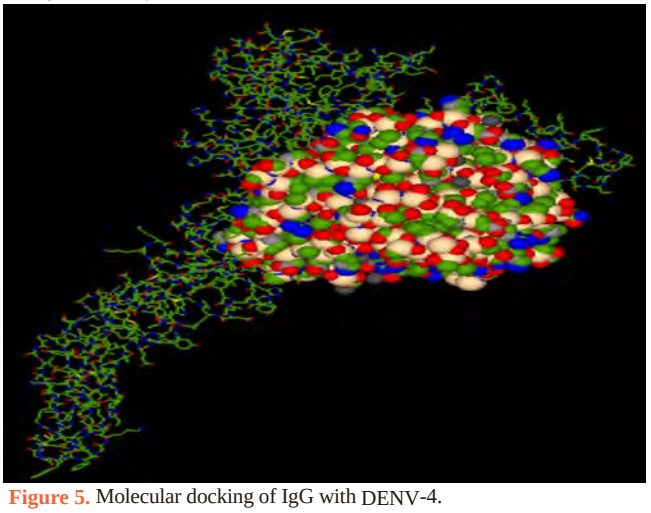

Figure 5. Molecular docking of IgG with DENV-4.

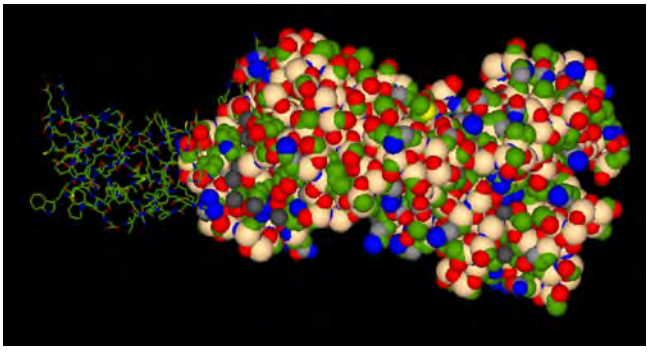

Figure 6. Molecular docking IgG with NS-1.

Table 2

Results of molecular docking (Hex) between antibodies of E and NS proteins.

Antibody	DENV-1	DENV-2	DENV-3	DENV-4	NS-1	NS-3	NS-5
IgA	-667.20	-604.50	-660.29	-630.55	-670.79	-641.56	-576.53
IgM	-689.22	-650.65	-660.05	-674.74	-591.00	-671.95	-684.93
IgG	-666.25	-602.38	-681.60	-699.03	-778.50	-693.62	-730.82

4. Discussion

Drug design is the inventive process of finding new medications based on the knowledge of a biological target[14]. The drug is the most common organic small molecule that activates or inhibits the function of a biomolecule such as a protein, which in turn results in a therapeutic benefit to the patient. In the most basic sense, drug design involves the design of small molecules that are complementary in shape and charge to the biomolecular target with which they interact and therefore will bind to it[15]. This type of modeling is often referred to computer-aided drug design. Finally, drug design that relies on the knowledge of the three-dimensional structure of the biomolecular target is known as structure-based drug design. Thus, the drug design is the design of a small molecule that

will bind tightly to its target[16]. Typically, a drug target is a key molecule involved in a particular metabolic or signaling pathway that is specific to a disease condition or pathology or to the infectivity or survival of a microbial pathogen. Some approaches are used to inhibit the functioning of the pathway in the diseased state by causing a key molecule to stop functioning. Drugs may be designed that bind to the active region and inhibit this key molecule. In the present investigation, various biomolecules, commonly used as drug molecules, were screened and identified through molecular docking as potential drug molecules for DENV serotypes.

Computational high-throughput docking has become a powerful tool for screening and identifying novel lead compounds. Computational approaches, not only save time and costs during screening, but also provide insight into understanding the molecular mechanisms of protein-drug interactions. The potential target, the molecule which is active sited on the receptor can be identified *in silico* by establishing the structure activity relationship of small molecules, ligands[17].

Computer-aided drug design uses computational chemistry to discover, enhance, or study the biologically active molecules. The most fundamental goal is to predict whether a given molecule will bind to a target and if so how strongly. Molecular dynamics are most often used to predict the conformation of the small molecule (ligand) with the biological target[18,19]. Serological testing of immunoglobulin (Ig) for dengue affected patients is an important investigation to identity the nature of the dengue fever, whether it is primary infection or secondary infection. As per the present study, IgM was recognized as Ig related to DENV-1 and DENV-2 indicating the primary infection. On the other hand, DENV-3 and DENV-4 were related with the production of IgG during secondary infection. The NS proteins also involved in the production of IgG. Much of the proteins' work has supported the present findings.

During a large outbreak of dengue serotype in Pakistan, serological testing for dengue IgM was performed. Dengue-specific IgM was detectable as early as 1 day, indicating the dengue fever caused by primary infection[20]. In the study on the dengue disease status in Tamil Nadu, India (2006-2008) by Gunasekaran *et al.*, IgM positively was recorded in the primary infection and IgG positively was denoted in the secondary infection[21]. In the docking studies, E proteins of DENV-1 and DENV-2 were highly interactive with IgM, while DENV-3 and DENV-4 were more interactive with IgG. All the NS proteins, NS-1, NS-3 and NS-5 showed effective interaction with the IgG. It is also concluded that DENV-1 and DENV-2 are IgM-positive and involved in the primary infection while DENV-3 and DENV-4 elicit IgG which is involved in the secondary infection.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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