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Potential Role of Recombinant Capsid Protein of Dengue Virus Serotype 2 in the Development of an In-House Elisa Based Detection of Infection

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SR, AA and MI planned the proposal of the study. RM, IA, SA and AV have contributed in the lab work. RM, IA, MS and MUK contributed in writing and editing of manuscript. All authors have read and approved the final manuscript.

Key words
Dengue virus serotype 2, Capsid gene (C), Cloning, Expression, Purification, ELISA

ABSTRACT

Dengue is an arboviral infection predominant in tropical and subtropical areas. It has inhabited the earth for the last few decades affecting almost 50% of the population in developing nations. Dengue infection covers a high range of symptoms for instance mild to acute hemorrhage fever, dengue shock syndrome, and liver dysfunction. Early diagnosis of the disease is crucial for prompt treatment strategies. One of the detection strategies of the infection can be made through viral structural peptides that act as markers for discernment, including Capsid (C) protein. In the proposed research, the structural gene obtained from local isolates was targeted for detection. For this purpose, a bacterial expression system was used to amplify, clone, and express the structural protein C. The expression of structural protein C was examined by SDS PAGE and verified by western blot and dot blot. The acquired antigen was purified which carries the potential for the development of a vaccine against dengue as well as for an in-house diagnostic assay. In this study, an in-house diagnostic ELISA has been compared to a commercially available kit to determine the specificity and sensitivity of the designed diagnostic ELISA. The aim underlying this study was to develop an accurate, error-free, unambiguous, and cost-friendly in-house ELISA, for the detection of antibodies in Pakistan's most prevalent dengue virus serotype 2 (DENV-2). The progress of this research would likewise foreshadow advancements of novel vaccines for future counteraction of dengue infection as C protein is highly immunogenic.

INTRODUCTION

Dengue infection caused by Dengue Virus (DV) is a mosquito-borne infection, typically observed in tropical and subtropical regions of the world (Khurram *et al*. 2021). DV belongs to the Flaviviridae family. Dengue is listed as most emerging vector born viral infection (Byk and Gamarnik, 2016; De Figueiredo *et al*., 2010). Almost 390 million dengue infections have been reported globally (Li and Kang, 2022). Over the last few decades, dengue cases have increased 30 times worldwide (Chavda *et al*., 2022). DV causes a complex set of medical symptoms ranging from mild infectious fever to fatal dengue shock syndrome (DSS) (Khurram *et al*., 2021). Dengue infection is divided into three stages mild fever infection, dengue hemorrhagic fever, and dengue shock syndrome (Rehman *et al*., 2022), the worst scenario occurs in the advanced stage of the disease that leads to dengue hemorrhagic fever (DHF), which has affected 5 million individuals globally (Stanaway *et al*., 2016). Dengue infected patients show different symptoms including high fever, headache, myalgia, skin allergies, and joint and muscle pains (Hasan *et al*., 2016).

Dengue infection has been responsible for immense deaths in more than 125 countries, with an estimated morbidity rate of approximately 10,000 deaths (Messina *et al*., 2019). Pakistan had its first dengue outbreak in 1994, however Pakistan had observed major outbreaks

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in 2006, 2010 and 2011 which affected more than 40,000 individuals (Ali *et al*., 2019). In 2020 almost 47,120 confirmed dengue cases were reported with 75 deaths (Rana *et al*., 2021). Similarly, in the year 2021, almost 48,906 confirmed dengue cases with 183 deaths have been reported in Pakistan (Islam *et al*., 2022).

DV has four different serotypes named DV-1, DV-2, DV-3, and DV-4. DV serotypes are classified based on morphology, antigenic characteristics, genetics, and replication behavior. Dengue viral genome is a positive-sense single-stranded RNA, packed in capsid (C) protein forming nucleocapsid (NC) (Gabriel *et al*., 2020). Dengue virus consists of a 50nm spherical glycoprotein shell composed of a lipid bilayer with two structural proteins envelop (E) and membrane (M) (Byk and Gamarnik, 2016; Macias *et al*., 2020). The glycoprotein shell consists of 180 copies of each E and M protein. The Pre-M is like a cap that covers the hydrophobic fusion part of E protein until the virus leaves the cell (Dwivedi *et al*., 2017).

Capsid (C) protein of DV is a highly basic protein having the size of 12KDa. C protein forms homodimers in solution. It has an association with both lipid membranes and the viral genome. It has a crucial role in nucleocapsid formation. C protein is responsible for the assembly of virion particles. It is the first gene of the dengue virus which has been translated. NC consists of one molecule of the viral genome and several copies of C protein. The procedure which C uses for the formation of NC is unclear yet (Byk and Gamarnik, 2016). Studies have shown that C protein is present in both the cytoplasm and nucleus of infected cells. In the nucleus of infected cells, C accumulates in the nucleoli, and in the cytoplasm, C protein is present on the membrane of ER and lipid droplets (Sangiambut *et al*., 2008).

The purpose of this study was to design an in-house diagnostic assay by producing antigens using a serological strategy. Later on this newly developed in-house ELISA kit was compared with a commercially available kit for dengue infection.

MATERIALS AND METHODS

Detection and serotyping of dengue

Dengue positive sera were collected from Peshawar laboratory during outbreak in 2017, and further examined for Dengue serotyping through nested PCR by using specific forward and reverse primers (Shahid *et al*. 2019). Serotyping of dengue samples was further confirmed by sequencing by using specific primers through 3100 Automated Genetic Analyzer (Applied BiosystemsInc). Positive dengue samples with serotype 2 (most prevalent serotype of dengue in Pakistan) were used for further study.

Amplification of dengue structural gene C

Dengue structural gene *C* of serotype 2 was amplified by using a specific set of forward and reverse primers. Capsid (C) gene was amplified by using forward primer as 5' CGCGCGGGATCCATGAATAACCAACGAAAAAAG 3' with *Bam*H1 restriction site at 5' and reverse primer as 5'CTGATTCCAACAGCGATGGCGTGACTCGAGCG-GGTC3' with *Xho*1 restriction site at 3'.

Cloning and sequencing

The amplified *C* gene was cloned in bacterial expression vector peT-28a. Amplified *C* gene was digested with *Bam*H1 and *Xho*1 restriction enzymes. The expression vector was also digested with *Bam*H1 and *Xho*1 restriction enzymes to produce sticky ends. The digested *C* gene was ligated to a freshly digested pET-28a vector. The recombinant plasmid was inserted and cloned in freshly cultured Top 10 bacterial cells for mass production of clone. The recombinant clone was checked by PCR using T7 forward primer and gene specific reverse primer for *C* gene. The constructed clone was further confirmed by double digestion analysis with *Bam*H1 and *Xho*1 restriction enzymes. The right orientation of insert into the vector was analyzed by sequencing using T7 promoter sequence as forward primer and gene specific reverse primer.

Bacterial transformation

The recombinant clone was transformed into bacterial competent Rosetta 2 DE3 cells for expression. The transformation was performed by using 1µl of recombinant plasmid containing gene insert for *C* gene in Rosetta bacterial cells and then subjected to heat shock for 60 seconds at 42°C. 0.9ml of LB media was added and incubated for 1 h at 37°C shaker. 50µl of transformant was spread on LB agar plate having two drugs kanamycin (50mg/ml) and chloramphenicol (50mg/ml) as double selection and incubated at 37°C overnight. Single isolated colonies were used for further expression of gene.

Expression studies

For expression analysis, single isolated colonies from plate were picked and inoculated in 5ml LB media containing both drugs chloramphenicol and kanamycin and incubated at 37°C overnight at the shaker incubator. Next day 1 ml culture was diluted in 20ml autoclaved LB media. The cells were allowed to grow at 37°C shaking incubator until optical density (OD) of cells reached approximately 0.6. At this stage, for the production of structural C protein, cells were induced with 1mM IPTG for 6 h at 37°C shaking incubator. After six h, cells were centrifuged at 5000rpm at 4°C for 10 min. The cell pellet was washed twice with 1X PBS. Cell pellet after induction

for 6 h, resuspended in 100µl 1X PBS. 2X loading dye with 2-mercaptoethanol was mixed with a sample. Samples were heat shocked at 100°C for 10 min for production of protein samples. After heat shock protein samples were placed on ice instantly. For expression analysis, protein samples were centrifuged at 14000 rpm at 4°C for 10 min. 10ul of supernatant containing crude proteins was used to run on 15% SDS gel for almost 1 h. Coomassie blue R250 dye was used to stain the gel for about 1 h. Then gel was de-stained through destaining solution overnight. The gel was visualized and the protein bands were determined by comparing pre-stained protein ladder.

Western blot analysis

For confirmation of recombinant protein, protein samples were subject to western blot analysis. Protein sample was run on 15% SDS-PAGE with a prestained protein marker. Required protein band of C was transferred to nitrocellulose membrane with the help of blotting papers by semi dry transfer method. For this purpose BioRad semi dry blotting apparatus was used. Required protein band was transferred to the membrane at 10V for about 50 min. After transfer, the membrane for protein was subject to blocking in blocking reagent which was 5% Bovine serum albumin (BSA) for 3 h. After blocking, the membrane was washed with PBST for 5 min vigorously three times. After washing the membrane was incubated with gene specific mouse monoclonal primary antibody. Anti C primary antibody (MA5-38247 Thermo-USA) was incubated with a membrane (having C protein band) in 1:1000 dilution at 4°C overnight. Next day the membrane was washed with PBST thrice for 5 min and the membrane was incubated with secondary antibody (SIGMA 017K6047, Anti-Mouse IgG) in 1:10000 dilution for 2 h at room temperature. Secondary antibody was conjugated with horseradish peroxidase (HRP). 3, 3`-diaminobenzidine (DAB) tablet by SIGMAFAST was mixed in 10ml PBS having 10µl hydrogen peroxide and solution was used as substrate. Membrane was treated with substrate for 20 min at 37 ºC, dried and image was captured.

Dot blot analysis

Dot blot analysis of C structural protein of dengue was carried out by using protein samples prepared by heat shock method. Nitrocellulose membrane was cut as strips for protein samples. Uninduced and induced sections were marked and labeled on the membrane. 1 µg of protein sample was poured in the center of the marked portion of the membrane for an induced and uninduced sample for C on the membrane, dried and then subjected to blocking. Membrane was blocked with BSA for 3 h. After blocking, the membrane was washed with PBST thrice for 5 min. After washing the membrane was incubated with gene specific mouse monoclonal primary antibody. Anti C primary antibody (MA5-38247 Thermo-USA) was incubated with a membrane in 1:1000 dilution at 4°C overnight. Next day the membrane was washed with PBST thrice for 5 min. Membrane was incubated with a secondary antibody (SIGMA 017K6047, Anti-Mouse IgG) in 1:10000 dilution for 2 h at room temperature. Secondary antibody was conjugated with HRP. DAB tablet by SIGMAFAST was mixed in 10ml PBS having 10µl hydrogen peroxide and solution was used as substrate. Membrane was treated with substrate for 20 min at 37ºC, dried and image was captured.

Protein purification

Required protein was purified through nickel affinity chromatography. The Ni-Sepharose column was used for purification of recombinant protein on AKTA Explorer. Protein samples were transferred to the columns and allowed to run. The columns were washed with a 1X PBS buffer. Unbound proteins were washed by 0.01M Imidazole, loosely bound proteins were washed through 0.5M Imidazole. Recombinant His conjugated His-C protein was eluted with 2M Imidazole.

Development of in-house diagnostic assay

A 96 well microtiter plate with flat bottom was coated with 100µl of purified antigen containing 70ng concentration of protein, with 100µl of 1X PBS overnight at room temperature. The plate was washed with PBS three times and subjected to blocking with a 5% blocking solution of BSA at 37°C for 2 h and then washed to remove blocking solution. After that, the plate was incubated with 100ul of dengue positive serum samples for each well at 37°C for 1 h. Then the plate was washed thrice with a washing buffer. After washing, the plate was incubated with a secondary antibody having HRP conjugated antibody to dengue antigen at 37°C for 1 h. After that, the plate was washed four times with 400µl of washing solution in each well. Almost 100µl TMB substrate solution was added in all wells and incubated the plate with cover in dark almost 18-24°C for 20 min. Stopping reagent containing 0.75M/L sulphuric acid was used to stop the reaction in each well and OD was measured for each well including positive and negative control at 450nm at Spectra Max Plus 384 spectrophotometer. The samples were run in triplicate and mean absorbance was plotted against antibody concentration. Patients optical density (O.D) values of both In-House ELISA kit and commercial kit were analyzed statistically by using a paired T-Test to find out any significant difference of results. All the analysis of data was performed by IMB SPSS version 22.0.

RESULTS

Amplified of capsid gene

Structural capsid gene of the DV was amplified by using a specific set of primers having restriction sites at 5' and 3'terminal. PCR product of C of 342bp (Fig. 1) was confirmed by sequencing analysis.

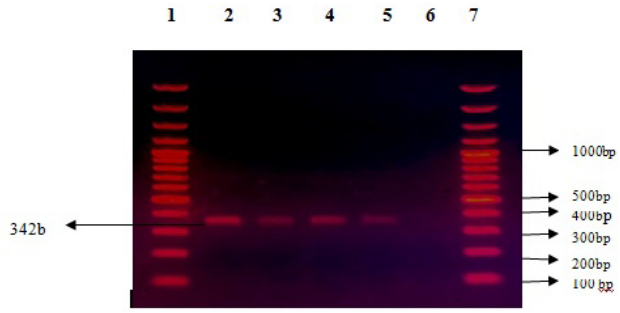

Fig. 1. PCR Amplification of dengue C Gene fragment, Lane 2-5: dengue DNA positive of 342bp and Lane 6 showing negative control (containing all PCR reagents but without Template DNA), Lanes 1 and 7: DNA marker of 100bp.

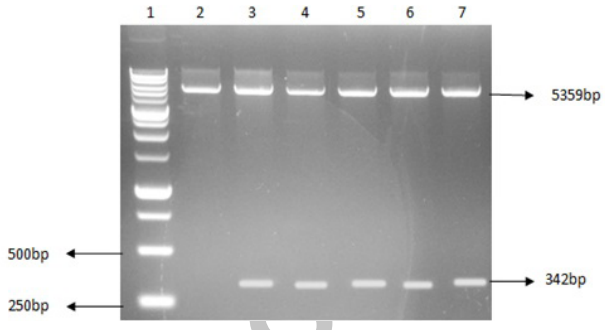

Fig. 2. Restriction digestion analysis of clone in expression vector: Lane 1: DNA marker of size 1Kb, Lane 2: negative control (empty vector), Lanes 3 to 7: First band digested of peT28a vector of size 5359bp and second band of dengue C gene fragment size 342bp.

Expression analysis of C *gene produce*

Amplified PCR product of *C* gene was purified by using PCR purification kit. Amplified product of C was cloned in peT28a vector and after digestion of both PCR product and vector with same restriction enzymes; ligation reaction was performed with T4DNA ligase. The clone of peT28a-C was confirmed by double digestion analysis. The results of double digestion were visualized in the form of bands on 1.5% agarose gel. Double digestion of peT28a-C had produced two bands on the gel sized 342bp and 5359bp (Fig. 2). The orientation of the construct was confirmed by using DNA Sanger's sequencing method. Clone was transformed into a bacterial expression system Rosetta 2 DE3. The expression of C was confirmed by SDS PAGE. Protein samples of induced and uninduced C were run on 15% SDS PAGE with a prestained protein marker for western blotting (Fig. 3). Western blot analysis showed a band of protein size 24KDa (capsid has size 12KDa but present in homodimer form) identified as AntiC-C conjugated protein as shown in Figure 4. The result of Dot blot of C has been shown in Figure 5.

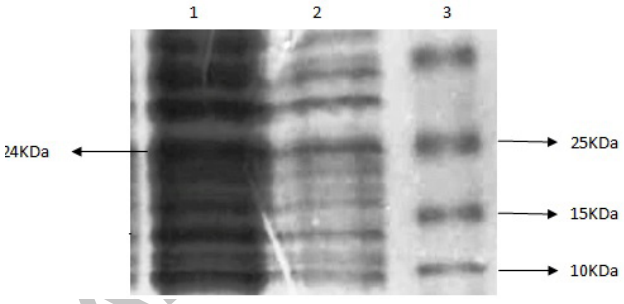

Fig. 3. SDS-PAGE of protein showing expression of 24KDa protein fragment. Lane 1: Induced protein sample, Lane 2: uninduced protein sample, Lane 3: Prestained Protein marker.

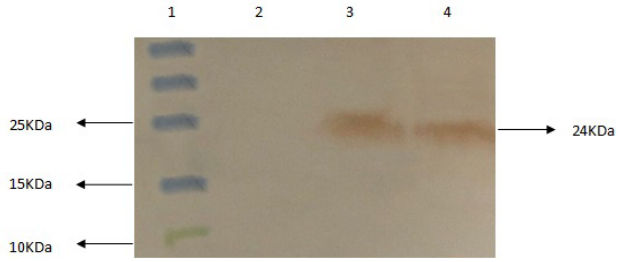

Fig. 4. Western Blot representing 24KDa protein fragment of C protein. Lane 1: Prestained Protein marker, Lane 2: uninduced protein sample, Lanes 3-4: Induced protein samples.

Antigenic response of antibodies against dengue

The antigenic response of antibodies against dengue was checked by using positive and negative serum samples with an already coated microtiter plate with purified recombinant C antigen by ELISA. Same protocol was performed with the microtiter plate of the commercial ELISA kit. Antibodies against dengue were identified in both in-house ELISA and commercial ELISA. Mean values and standard deviation (calculated from three OD values of each sample for all 24 samples) of commercial ELISA and in-house ELISA have been presented in Tables I and II, respectively. A paired T-test was applied to find

any significant difference between patients' O.D values for the In-House kit and the commercial kit. The results showed no significant difference (P-value = 0.59).

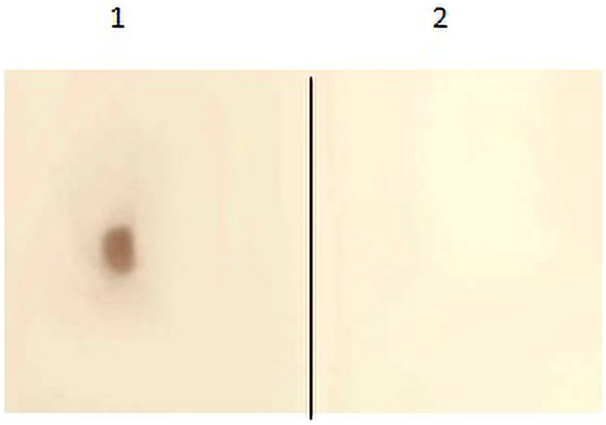

Fig. 5. Dot blot representing C protein of dengue. Lane 1: Induced protein sample, Lane 2: uninduced protein sample.

DISCUSSION

Pakistan has been facing major outbreaks of viral infections resulting in massive deaths over the last few decades (Narayan *et al*., 2016). Dengue is the most prevalent vector-borne viral infection in Pakistan. Currently, studies have reported co-infection of Covid and Dengue. This co-infection has put more burdens due to viral outbreaks and also strained out our health care system. Compromised poor healthcare aptitude, socioeconomic disparities, low vaccination rate, and misinformation have made the condition more miserable for the Pakistani population (Islam *et al*., 2022). Therefore, an early and accurate viral detection is incredibly important. Previously, diagnosis of infection has been done by virus isolation (NAAT nucleic acid amplification tests) and serological testing which include antigen and virus-specific antibodies detection. Studies demonstrated that viral nucleic acid isolation has been proven to be more pinpointed and sensitive to detect infection than serological methods but the facility of these tests is low, due to higher costs particularly, in developing countries like Pakistan (Organization) (Islam *et al*., 2022). However, serological tests include IgM/ IgG-based, antibodies testing and NS1-based antigen testing. Mainly E/M based IgG and IgM ELISA and NS1 antigen has been used for serological detection of dengue infection, but later on, studies have reported that anti-C antibody can be used to detect early dengue infection by quantifying recombinant C protein in an antigen capture Enzyme-Linked Immunosorbent Assay (ELISA), suggesting future of C protein in early diagnosis of dengue infection.

Table I. Mean and standard deviation values of commercial ELISA.

S. No	Value 1	Value 2	Value 3	Mean ± SD
1	0.28	0.78	0.285	0.28 ± 0.287
2	0.231	0.201	0.174	0.202 ± 0.028
3	0.236	0.246	0.259	0.247 ± 0.011
4	0.236	0.283	0.291	0.27 ± 0.029
5	0.275	0.244	0.213	0.244 ± 0.031
6	0.081	0.11	0.168	0.12 ± 0.044
7	0.271	0.201	0.291	0.254 ± 0.047
8	0.285	0.293	0.301	0.293 ± 0.008
9	0.311	0.291	0.261	0.287 ± 0.025
10	0.3	0.291	0.261	0.284 ± 0.020
11	0.213	0.281	0.261	0.251 ± 0.034
12	0.2	0.193	0.196	0.196 ± 0.003
13	0.251	0.298	0.251	0.266 ± 0.027
14	0.224	0.2	0.261	0.228 ± 0.030
15	0.226	0.26	0.243	0.243 ± 0.017
16	0.2	0.231	0.26	0.237 ± 0.030
17	0.29	0.291	0.287	0.289 ± 0.002
18	0.298	0.29	0.287	0.291 ± 0.005
19	0.261	0.263	0.265	0.263 ± 0.002
20	0.198	0.185	0.195	0.192 ± 0.006
21	0.241	0.25	0.213	0.234 ± 0.019
22	0.234	0.26	0.243	0.245 ± 0.013
23	0.289	0.28	0.279	0.282 ± 0.005
24	0.198	0.199	0.213	0.203 ± 0.008
Positive control	0.105	0.193	0.284	0.194 ± 0.089
Negative control	0	0.0658	0.047	0.0235±0.033

ELISA assays have proved to be cost-effective. A diagnostic assay based on IgM ELISA has proved ~ 90% sensitive and 98%, specific if sera were collected five days or later after the onset of illness. Quality of antigen has a great impact on the sensitivity and specificity of IgM-based diagnostic assay and variation of sensitivity and specificity of IgM-based ELISA between different commercial kits has proved it (Peeling *et al*., 2010).

In various studies, antibodies of C proteins were detected in many dengue-positive patients' sera (Puttikhunt *et al*., 2009) have reported that dengue serotype 2 capsid monoclonal antibody can be used to detect early dengue infection. Purified C protein can be used as a diagnostic tool (Selvarajah *et al*., 2012) although it is not exposed to the dengue virus surface. Monoclonal antibodies against dengue 2 C protein has been shown low cross-reactivity

against other dengue serotype C proteins (Noda *et al*., 2012). In our study we designed in house diagnostic ELISA based on antibody detection in patients' sera using purified recombinant C antigen which is specific to Pakistani isolate, showing more specificity and sensitivity to local isolate.

Table II. Mean and S. deviation values of in-house ELISA.

S. No	Value 1	Value 2	Value 3	Mean ± SD
1	0.284	0.264	0.293	0.28 ± 0.014
2	0.257	0.27	0.283	0.27 ± 0.013
3	0.277	0.278	0.284	0.28 ± 0.003
4	0.3	0.315	0.328	0.314 ± 0.014
5	0.274	0.313	0.357	0.315 ± 0.041
6	0.0093	0.1967	0.085	0.097 ± 0.094
7	0.261	0.198	0.199	0.219 ± 0.036
8	0.219	0.281	0.267	0.255 ± 0.032
9	0.298	0.285	0.261	0.281 ± 0.018
10	0.301	0.311	0.268	0.293 ± 0.022
11	0.211	0.293	0.285	0.263 ± 0.045
12	0.211	0.198	0.267	0.22 ± 0.036
13	0.268	0.3	0.281	0.283 ± 0.016
14	0.223	0.216	0.281	0.24 ± 0.035
15	0.234	0.267	0.251	0.25 ± 0.016
16	0.216	0.238	0.269	0.241 ± 0.026
17	0.298	0.293	0.287	0.292 ± 0.005
18	0.312	0.298	0.281	0.297 ± 0.015
19	0.268	0.271	0.278	0.272 ± 0.005
20	0.193	0.199	0.201	0.197 ± 0.004
21	0.244	0.254	0.222	0.24 ± 0.016
22	0.243	0.267	0.251	0.25 ± 0.012
23	0.291	0.285	0.286	0.287 ± 0.003
24	0.199	0.201	0.243	0.214 ± 0.024
Positive control	0.241	0.245	0.276	0.25 ± 0.019
Negative control	0.047	0.047	0.049	0.048 ± 0.001

Recombinant C protein has been reported as an important immunogen in vaccine development as well. Studies reported that the C protein stimulates a strong antibody response in dengue-infected human sera (Nadugala *et al*., 2017). According to a recent study, the C protein of the dengue virus is a very important antigen that elicits a strong immune response and acts as epitopes for T cells and B cells (Dixit, 2021). Different bioinformatics and immunoinformatics tools are used to identify epitopes of C protein for T and B cells (Tong and Ren, 2009).

Table III. A, Descriptive analysis of O.D values of two kits (PanBio and in-house ELISA). B, comparison of O.D values in PanBio and house ELISA kit (Paired T-test).

(A)	Mean ± SD	N	SEM		
PanBio ELISA	0.245±0.049	24	0.008		
In-House ELISA	0.256±0.046	24	0.009		
(B)	**Mean difference**	**T statistic**	**N**	**Mean ± SEM**	**P value**
PanBio ELISA vs in-house ELISA	-.010375	-1.990	23	.005	0.059

Because of the viable role of recombinant proteins in diagnosis and therapeutics, different expression system has been used. However, commercial success lies in the production of massive recombinant proteins at a low cost. Scientists have been using different expression systems, in which *Escherichia coli* in the prokaryotic system has been widely used. *E. coli* has been the prime choice for the expression of recombinant proteins, as about 30% of biopharmaceuticals and 70% of proteins for research purposes are produced by using *E. coli* expression system (Magalhães *et al*., 2022). In this study, the prokaryotic expression system is used in comparison to the eukaryotic system as it is simple, prompt, and cost-friendly. In previous times, the prokaryotic system especially *E. coli* has been used for the production of recombinant C protein with *Bam*H1 and *Xho*1 restriction sites (Puttikhunt *et al*., 2009). Recently, a study reported that the C protein of dengue 2 has been cloned in peT3a and expressed in the *E. coli* expression system (Mebus-Antunes *et al*., 2022). As C protein has a crucial role in the viral life cycle and infect host cells, its expression and purification can be helpful in the diagnosis of the infection and designing future vaccines and therapeutics (Zhang *et al*., 2021).

In our study, we designed an in-house diagnostic assay and compared it with a commercially available diagnostic kit named PanBio. Previously, such comparison has been made in different studies, Chong *et al*. (2021) had compared their diagnostic assay (ViroTrack Dengue Serostate, a biosensors-based semi-quantitative anti-dengue IgG immuno-magnetic agglutination assay) with PanBio Dengue IgG indirect and capture enzyme-linked immunosorbent assays for the detection of previous and recent dengue infection (Chong *et al*., 2021), biotinylated-EDIII-based immunoassay has been developed and compared with commercial PanBio kit for surveillance purpose (Yadav *et al*., 2022), in different

study commercial ELISA kits Calbiotech and PanBio have been compared with RT-PCR, statistics have been shown that PanBio ELISA sensitivity more significant related to PCR (Khan *et al*., 2009). Ansari *et al*. have been used the PanBio dengue diagnostic kit in their study to find out the association between the severity of the disease with IgG level in confirmed dengue-positive cases in a secondary infection (Ansari *et al*., 2021). Guzman *et al*. (2010) have made a comparison (in 30 studies from different countries) between NS1-based ELISA from two commercially available kits PanBio and Platelia (Guzman *et al*., 2010).

The present study proves to be an initial step toward the development of an in-house cost effective and sensitive method of infection detection.

CONCLUSIONS AND RECOMMENDATIONS

In this study, recombinant Dengue-2 C has been expressed and purified by using nickel Ni-affinity chromatography. The purified C protein can be the best candidate for the designing of diagnostic assays and therapeutic vaccines against dengue. The purified C antigen was utilized to detect the anti-capsid antibodies in the dengue-positive serum samples by in-house diagnostic ELISA. Same samples were used for the detection of antibodies with a commercial kit and a comparison of both kits was drawn. The in-house designed kit proved to be more specific and cost-effective against the screening of local samples. The cost of a commercial kit per sample was 2.1 USD and the cost of screening samples by local kit was 0.02 USD approximately. This will help our country with limited health support to share the disease burden of dengue outbreaks each year.

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IRB approval

The Punjab University Institutional Ethics Review Board approved the study vide letter number D/78/FIMS

Ethical statement

A written consent letter signed from each patient was obtained from all the patients before sample collection of this study.

Statemenet of conflict of interest

The authors have declared no conflict of interest.

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