

RESEARCH ARTICLE

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Computational Approach for Predicting Common Epitopes in the VP1 Structural Protein of Enterovirus Serotypes EV-D68, EV-D70, and EV-A71

Rihabe Boussetine , Yassine Kasmi , Najwa Hassou ,
Hlima Bessi and Moulay Mustapha Ennaji* 

Laboratory of Virology, Microbiology, Quality and Biotechnologies/Ecotoxicology and Biodiversity Faculty of Sciences and Techniques Mohammedia University Hassan II of Casablanca, PB 146 Mohammedia (20650). Morocco.

Abstract

The three human Enterovirus serotypes D-68, D-70, and A-71, are common pathogens that are transmitted by fecal-oral and aerosol routes. These positive RNA viruses were known to exhibit high levels of genetic diversity and variability. Currently, no vaccines are available to protect humans from these three serotypes. Therefore, efforts are needed for the development of a vaccine directed against heterologous viruses. In our study, an immunoinformatics approach is used to identify T- and B-cell epitopes that may help for the generation of a universal vaccine against EV-D70, EV-A71, and EV-D68. B and T cell epitopes were selected based on their length. As a result, 5 B cell epitopes and 18 T cell epitopes were predicted. Our B cell epitope prediction results showed that there are a number of linear regions. Position 150-170 was found to be the most immunogenic for the different strains. Regarding the epitopes of the T lymphocytes, the result of the interactions shows that 95% of the predicted epitopes are common between the 3 sequences and the 5 methods used. These results demonstrate the great immunogenic potential of these sequences and their capacities to trigger immune reactions in people with different HLA alleles. The "VFYDGFAGF" epitope is the most important and most immunogenic for triggering an immune response. Our study results allowed us to identify epitopes to be used in the development of cross-protection vaccines against the three Enterovirus serotypes. However, in vivo and in vitro studies are needed to assess the potential of the epitopes predicted by our study.

Keywords: Enterovirus, Epitopes, VP1 structural protein, Computational prediction

*Correspondence: m.ennaji@yahoo.fr.

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INTRODUCTION

Human enteroviruses (EV) EV-D68, EV-D70, and EV-A71 belong to the genus *Enterovirus* of *Picornaviridae*.¹ They are highly resistant to external environmental conditions. Their transmission mode is mainly faecal-oral, although they can also be transmitted by aerosols.² There does not appear to be a relationship between a given genotype and a particular geographic area.³ *Enteroviruses* (EVs) are common infectious agents and they currently comprise 108 serotypes. They are non-enveloped, single-stranded, positive-polarity RNA viruses, characterized by a genome size of approximately 7500 nt that codes for 11 viral proteins. Their capsid consists of 60 identical protomers, or capsomers, each containing 4 structural viral proteins (VP) such as: VP1 (34 kDa), VP2 (30 kDa), VP3 (26 kDa), and VP4 (7 kDa). VP1, VP2, and VP3 form a wide depression of 15 Å on the surface of the virus, called a canyon, which is thought to be the interaction site of the virus and its receptor.^{4,5}

The VP1 protein encoded by the 1D protein gene is largely exteriorized on the surface of the virion and it bears several antigenic epitopes located mainly in the peptide loops that connect the beta-sheets.⁶ The VP1 protein of EV-D68, EV-D70, and EV-A71 is involved in attachment of the virus to the host cell,⁷ not only as a major structure of the canyon site but also due to its antigenicity, as it bears a set of epitopes involved in specific recognition of these viruses.⁸

In light of the pathogenicity of EVs, the currently available therapeutic means are very limited or non-existent in clinical practice.⁹ The development of new molecules and/or antiviral strategies is hence a necessity and should lead to greater knowledge of the infection cycle of the target cell, in particular, the processes of attachment and entry of the virus into the cell.¹⁰ Given the technological potential of subunit vaccines compared to conventional whole virus vaccines, we investigated the benefit of using the VP1 subunit of enteroviruses is sufficient to obtain adequate protective immunity. Due to the antigenic importance of the three-dimensional structure of viral capsids in which all the capsid proteins function in concert, the application of VP1 subunit vaccines for enteroviruses has always been reluctant, despite the fact that the agglutination

of many epitopes on VP1 independent of other capsid proteins has been widely demonstrated.¹¹ In our research, we used an immunoinformatics approach based on computational approaches for predictive vaccines in order to select common epitopes for the three serotypes to evaluate the conservation of the peptide sequences of EV-D68, EV-D70, and EV-A71 at the level of the VP1 protein using reference sequences from the NCBI genome bank. Subsequently, linear epitopes of B lymphocytes and T lymphocytes were predicted and their accessible surface identified as well as their ability to generate an immune response.

The analysis of the results obtained will be used to consider a therapeutic treatment that can contribute to a reduction of infections caused by these three enteroviruses.

METHODOLOGY

In this work, we used modeling tools in order to study and determine the potential epitopes of VP1 that can be used for the purpose of vaccination against EV-D68, EV-D70, and EV-A71.

Data set

The following amino acid sequences of VP1 of serotypes A, B, and C were obtained from the National Biotechnology Information Centre (NCBI) database: 183 amino acids of VP1 of EV-D68 (AYG85340.1), 310 amino acids of VP1 of EV-D70 (BAA08157.1), and 299 amino acids of VP1 of EV-A71 (ACS12928.1).

Sequences

Different genotypic protein sequences of enterovirus protein VP1 were obtained from the National Biotechnology Information Centre (NCBI). Complete sequences of all subtypes of different genotypes were selected. Sequences containing only partial sequences of genotypes were excluded.

After obtaining the NCBI protein sequences, multiple sequence alignments were performed using Muscle MegaX software. The default settings were used on the interface. The highest numbers of similar and identical amino acids without gaps covering the protein sequence were selected as conserved areas. These conserved areas were used to predict B-cell linear epitopes, surface-accessible epitopes, and antigenic sites.

Protein structure Prediction and consensus sequence validation

Subsequently, the reference sequences for VP1 of genotypes A, B, and C were submitted to the Swiss-Modeler server¹² for the prediction of the homology degree of the 3D structure. Three-dimensional proteomic structures with a greater than 80% identity and that had a statistically significant homology (e-value 0.001) were selected. The sequences for which these conditions do not apply were modeled with the I-TASSER¹³ server in order to predict the 3D structure of the protein using an *ab initio* computational approach that can also predict their biological function. The consensus sequence was uploaded to the I-TASSER server for thread structure prediction (<http://zhanglab.ccmb.med.umich.edu/I-TASSER>). The planned structure was refined by ModRefiner.¹⁴ The quality of the structure was confirmed by the Ramachandran plot.¹⁵

Epitopes prediction

The B- and T-cell epitopes prediction of was made using methods that differ from the common B- or T-cell epitopes, among all of the methods used, and were determined via

- For “T”-cell epitopes

The tools offered in IEDB were used. Before predicting the epitopes, each epitope was submitted to analysis by the following algorithms: BIMAS,¹⁶ SYFPEITHII,¹⁷ and NetMHC.¹⁸

Epitope prediction tools were used to check if the epitopes can be presented on the surface of the cells, The T-cell epitope prediction tools were used to predict the potential epitopes of the EV-VP1 protein: MHC-I, MHC-NP, NetCTLpan 1.1, RANKPEP,¹⁹ and NetMHCpan 3.0 treatment predictions. Since HLA-A0201 was the most common CMH allele and most of the MHC-I epitopes were monoepitopes, the allele and monoepitope HLA-A0201 were selected. The other parameters of each prediction tool were set by default.

The purpose of the MHC-I IEDB treatment prediction server is to identify CMH-I ligands. This website provides a tool for the prediction of protein-treated peptides naturally presented by MHC Class I molecules. The MHC-I linkage forecast was established on March 30, 2019, using the consensus of the IEDB analysis resource, which combines the forecasts of ANN, aka NetMHC (3.4),

SMM, and Comlib (<http://tools.immuneepitope.org/processing/>).

The MHC-NP server²⁰ is developed for the prediction of peptides naturally presented by CMH molecules. This website uses data from CMH elution experiments to assess the probability of a given peptide being treated naturally and its binding to a given CMH molecule (<http://tools.immuneepitope.org/mhcnp/>). The NetCTLpan 1.1 server is designed to predict the epitopes of cytotoxic lymphocytes (CTL) in protein sequences. This website integrates the predicted link to the MHC-I peptide, the cleavage of the C-terminal proteasomal cleavage site using artificial neural networks (ANN),²¹ and the transport efficiency of the TAP (protein), which was predicted using the weight matrix (<http://www.cbs.dtu.dk/services/NetCTLpan/>).

The purpose of the RANKPEP server is the prediction of the epitopes of CMH Class I and Class II molecules based on protein sequences. This method predicts epitopes using position-specific score matrices (PSSM) (<http://imed.med.ucm.es/Tools/rankpep.html>). The NetMHCpan3.0 server is designed to predict the peptide-MHC binding capacity of Class I.

- For the “B”-cell epitopes prediction:

Four different methods were used to predict the B cell epitopes: HMM (Hidden Markov Model), ANN (artificial neural network),²¹ and deep learning methods.²² We defined the threshold of positive epitopes if they are equal or bigger than 1.0 in the Immune server package Epitope DataBases (IEDB),²³ The epitopes were validated by the method of Chou and Fasman beta turn.

RESULTS

VP1 3D models and alignments

Although that in PDB database, there is numerous crystallized structures from the complete virus. The screening condition to select the protein were fall done due to the Bigger Resolution which is often bigger than 3 Å. In addition, as we would like to include the mutation observed in the sequences recently published. We used the *ab initio* approach to model 3D structures and to optimize structures via ModRefined (Fig. 1). The published structure in PDB were almost similar to our modeling results with a few modifications due to the mutations recently revealed.

VP1 “T”-cell epitopes

In a pre-selected environment, the IEDB recommends MHC-NP, NetCTLpan 1.1, RANKPEP, and the NetMHCpan 3.0 server to predict powerful epitopes of the sequence. In order to improve the accuracy of epitope prediction, epitopes predicted by at least four tools have been selected. (the full prediction of epitopes is included in the Supplementary Data). The analyses showed that HLA-A*01:01 has greater predictive value for the 3 sequences.

The result of the interactions shows that 95% of the predicted epitopes are common between the 3 sequences and the 5 methods used. These results thus demonstrate the great immunogenic potential of these sequences and their capacities to trigger immune reactions in people with different alleles. However, it should be pointed out that the immunogenic rate differs between the sequences and the epitopes. The table above presents the list of the 18 epitopes predicted for VP1 T lymphocytes.

Epitope “VFYDGFAGF” is the most important and immunogenic one, with a percentage of 90% and an IC of 28 nM to trigger an immune reaction. However, the other epitopes require more than 100 nM. This counts better for several reasons including TAP and MHC. Other shared epitopes of the same importance are

shown in the Supplementary data. “SSN” epitopes get a response on 225 predicted positions. An advanced understanding of antigen recognition at the molecular level enables the development of an epitope vaccines. The approach to building an epitope vaccine is based on the identification of B-cell epitopes and T-cell epitopes. These epitopes are immunodominant and have the capacity to induce a specific immune response.

VP1 “B”- cell epitopes

For B epitopes, linear epitopes were identified by the HMM and ANN methods, which showed that there are a number of linear regions. The position 150-170 was found to be the most immunogenic for the different strains (Fig. 2)

DISCUSSION

Enteroviruses are responsible for several diseases that adversely impact public health, notably neurological manifestations: lymphocytic meningitis and flaccid paralysis (EV-D70 and EV-A71)^{24,25} skin and mucosal membrane manifestations: hemorrhagic conjunctivitis, MPB syndrome (EV-A71)²⁶, and rhinopharyngitis and respiratory infections: bronchiolitis (EV-D68).²⁷

The EV capsid VP1 protein is one of four structural proteins of EV. Since the VP1 protein is highly exposed and has been reported to play an important role in viral pathogenesis and

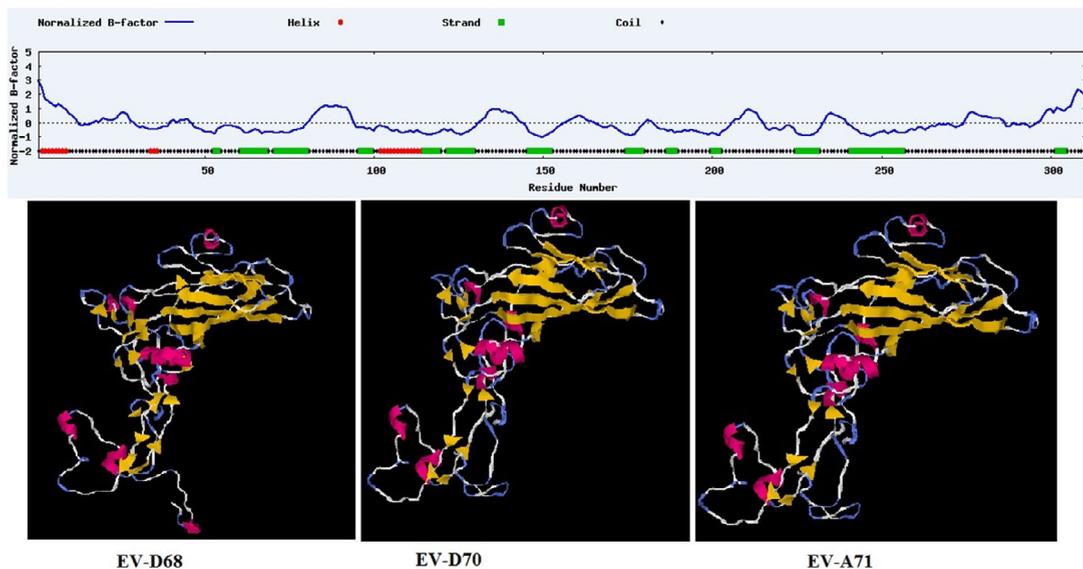


Fig. 1. 3D image of the VP1 structural protein of EV (from I-TISSAR; 13).

Table 1. List of 18 predicted epitopes common for T lymphocytes of the three serotypes (EV-D68, EV-D70, EV-A71)

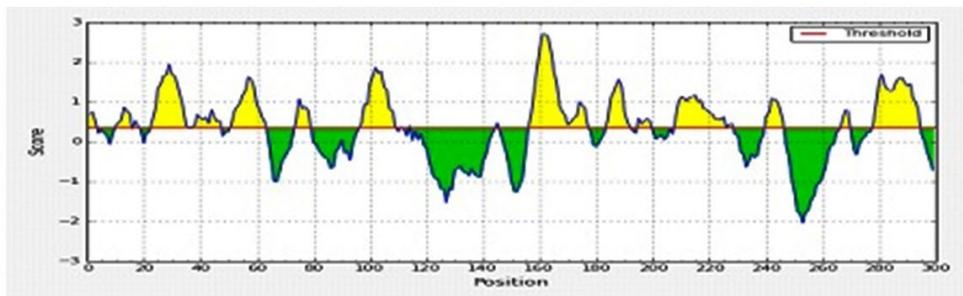
Allele	Start	End	Peptide Length	Peptide	Proteasome score	TAP score	MHC score	Processing score	Total score	MHC IC50 (nM)
HLA-A*01:01	266	274	9	YTNILNINNY	1.39	1.15	-1.41	2.54	1.13	25.5
HLA-A*23:01	175	183	9	VFYDGFAGF	1.31	1.30	-1.42	2.62	1.19	28.0
HLA-A*02:06	174	183	10	SVFYDGFAGF	1.31	1.21	-1.88	2.52	0.65	75.3
HLA-A*01:01	266	275	10	YTNILNINNY	1.20	1.15	-1.26	2.35	1.08	18.3
HLA-A*24:02	234	242	9	SYQPVQYTL	1.60	0.60	-1.16	2.20	1.05	14.3
HLA-A*24:02	197	205	9	AYANFYDGF	1.35	1.31	-1.61	2.66	1.04	41.1
HLA-A*24:02	196	205	10	SAYANFYDGF	1.35	1.29	-1.88	2.64	0.76	75.2
HLA-A*24:02	132	140	9	LFSSNVSF	1.55	1.23	-2.15	2.78	0.63	142.4
HLA-A*02:06	26	34	9	GVIPSLNAV	1.19	0.17	-0.78	1.36	0.58	6
HLA-A*11:01	193	202	10	SINSAYANFY	1.33	1.32	-2.17	2.66	0.49	147.2
HLA-A*24:02	131	140	10	RLFSSNVSF	1.55	1.28	-2.39	2.84	0.45	243
HLA-A*24:02	111	120	10	KMELFTYLR	1.37	1.19	-2.13	2.56	0.44	134.1
HLA-A*01:01	192	202	11	MSINSAYANFY	1.33	1.29	-2.21	2.62	0.42	160.8
HLA-A*02:01	117	130	14	YLRFDTEITVPTL	1.74	0.44	-1.79	2.19	0.40	61.5
HLA-A*11:01	72	81	10	ALVCMRSFEY	1.30	1.40	-2.36	2.70	0.34	228.9
HLA-A*11:01	262	275	14	RTMPYTNILNINNY	1.20	1.33	-2.20	2.53	0.33	157
HLA-A*02:06	262	270	9	RTMPYTNIL	1.73	0.48	-1.87	2.21	0.33	74.8
HLA-A*11:01	73	81	9	LVCMRSFEY	1.30	1.40	-2.39	2.71	0.31	246.3
HLA-A*26:01	222	235	14	NTMGNLCLRVVNSY	1.28	1.37	-2.40	2.66	0.25	252.6
HLA-A*02:01	117	126	10	YLRFDTEITI	1.32	0.26	-1.33	1.58	0.25	21.3

virulence,²⁸ and its antigenic homology among many different EV serotypes has been well documented.²⁹ The N termini of most EV VP1 proteins contain highly conserved immunogenic regions that are recognized by sera from most EV-infected patients.³⁰ This results suggests the potential use of the EV VP1 protein as a target to raise pan-EV mAbs (monoclonal antibodies) for the detection of a broad spectrum of EVs.

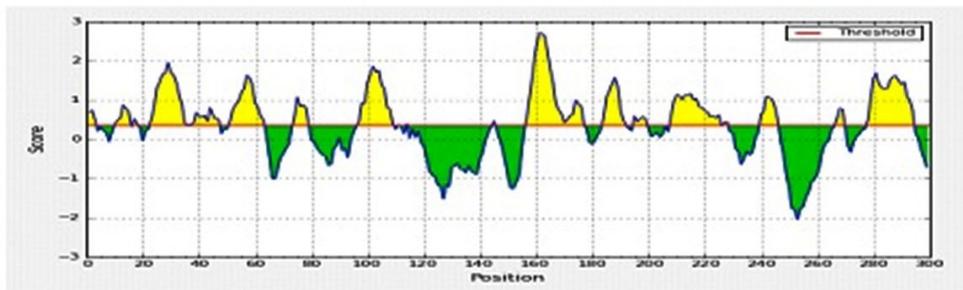
To make the molecule immunogenic, B-cell epitopes may be linked to T-cell epitopes. Advances in bioinformatics have had significant

impact in immunology sector, which allowed the rapid development of computer immunology also called immunoinformatics

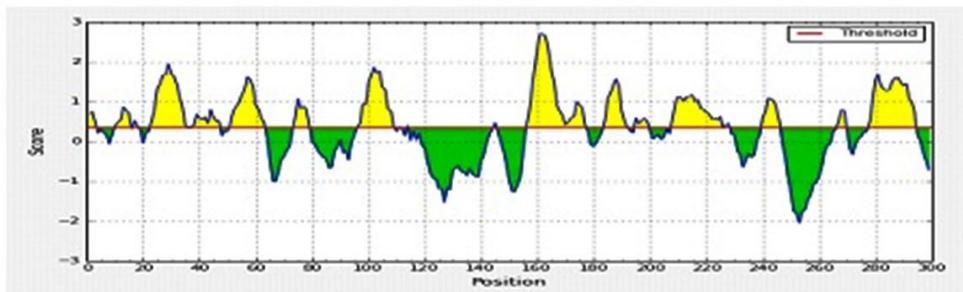
Immunoinformatics research considerably assists with mapping T and B epitopes of pathogens. Using various bioinformatics tools, immunologists are able to analyze potential sequences and their linking sites in a short time frame. This can lead to the development of new efficient vaccines. In this research, various bioinformatics algorithms to develop an effective multi-epitope vaccine were used. In addition to the existence of 68 viral



A: Prediction of B cell epitopes for D1, EV-A71



B: Prediction of B cell epitopes for D1, EV-D68



C: Prediction of B cell epitopes for D1, EV-D70

Fig. 2. Results of prediction of B linear epitopes common by HMM and ANN of the three serotypes (EV-D68, EV-D70, EV-A71).

serotypes, the antigenic properties change even within a given strain.

The conserved areas were analyzed for the identification of B-cell epitopes, antigenicity of the epitopes, accessibility to the surface, conservation of the epitopes, and hydrophilia in order to select the final epitopes of B cells. In addition, the conserved regions were analyzed and T-cell epitopes were also identified. The epitopes predicted from the conserved areas exhibited all of the properties described above. The Immune Epitope Database (IEDB) (<http://tools.immuneepitope.org/bcell/>) was used to predict antigenic sites. Kolaskar and Tongaonkar antigenicity methods were used for to predict antigenic sites with a default threshold value of 1.0. The B-cell epitopes were predicted using the consensus sequence. The prediction of T-cell epitopes were made using alleles for haplotypes A18, A31, A19, A14, A20, A11, A13, and A10 in which the Class I HMC genes were expressed. Selection of the epitopes was carried out and the duplicate epitopes were removed. The selection of B and T cells epitopes were based on length. As a result, 5 B lymphocyte and 18 T lymphocyte epitopes were predicted.

Novelty of this work lies in the evaluation of the possibility to synthesize a common vaccine for the three serotypes, although other work may have highlighted vaccines for EV-A71 or EV-D70.

Synthetic peptide vaccines designed to achieve specific immune responses against pathogens have several advantages. First, they are relatively simple and cost-effective to produce, and secondly, a large variety of peptide vaccines can be synthesized.³¹ While this area of research is not yet well established, a lot of work is currently being carried on synthetic peptide vaccines.

In the same line with our results, the study conducted by Park et al³² regarding the development of a synthetic vaccine for several EV-D70 strains showed that E peptide-induced antibodies should protect against most EV-D70 strains, and their results highlight the potential of the E peptide as a synthetic peptide vaccine against EV-D70, while the results from the study of Chen et al³³ demonstrated that an N-terminal fragment (aa 1–138) of EV-D70 VP1, expressed by a bacterial expression system, induces more neutralizing antibodies than a C-terminal fragment (aa 141–

310). These studies corroborate our results. Chen et al³³ showed that the N- and C-terminal fragments have independent antigenic neutralization sites, which is consistent with our results, provided that the B epitopes that we predicted are located in the same regions. Therefore, EV-D70 VP1 appears to have several independent neutralizing epitopes. Particularly important is the observation that the E-peptide region in the N-terminus of VP1 is a powerful B epitope that can induce the production of neutralizing antibodies against EV-D70. Thus, it could be a possible target for the development of an effective vaccine against EV-D70 peptides.³²

Another study, by,³⁴ described two subunits of EV-A71 vaccines, one as a DNA vaccine and the other as a recombinant protein vaccine that induced a neutralizing antibody response in both ICN and BALB/c mice. These results provide evidence that VP1 of EV71 contains neutralizing epitopes independent of other viral capsid proteins and lead the way for the potential use of VP1 as a basic antigen for the development of EV-A71 subunit vaccines.

The special form of the antigen is generally considered to be a better independent activator of T and B lymphocytes^{35,36} compared to the soluble antigen. Nevertheless, if these mechanisms works in EV-A71, immunity should be studied empirically.³⁴ These results were verified by our study, which led to the prediction of B epitopes but also 18 T epitopes that are immunodominant and that have the ability to induce a specific immune response.

However, for EV-D68, the work by³⁷ has shown that, since the first virus isolation, mutations have been observed in the BC and DE loops of the VP1 sequence, which corresponds to the region of the protein on the viral surface linked to antigenic epitopes, therefore indicating that unique sequence variations in these loops may cause a change in antigenicity, although there is little data on serotype vaccination in the current literature. Therefore, our study adds significantly in this regard.

CONCLUSION

This *in silico* study represents a major step forward in the development of a common vaccine against viruses that present a major risk to public health. However, the feasibility of such vaccine

development must be verified by in vivo studies. As a better way to use the results of this study, this work can be considered as a basis for in vivo studies.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

BR, HN, BH, MME conceptualized the project. BR, KY, HN proposed the methodology. BR, KY, BH, MME validated the results. BR wrote the manuscript. HN reviewed the manuscript. All authors read and approved the manuscript for the final publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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