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In Silico Characterization of Epitopes from Human Rotavirus VP7 Genotype G9 Design for Vaccine Development

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ABSTRACT

Acute gastroenteritis caused by Rotavirus remains the leading cause of child mortality worldwide. Rotavirus genotype G9 circulates in humans throughout the world. Antibodies against the outer glycoproteins VP7 and VP4 Rotavirus capsid are the main neutralization antibodies in the vaccine assessment. This study aimed to select an epitope to evoke T and B cells' response, as a favorable candidate for vaccine development using in Silico evaluation.

In the present study, Rotavirus genotypes were determined in 100 stools specimens collected from children with acute diarrhea. The results showed predominant G genotype, G9 (38.5%) followed by G2 (22.9%), G1 (16.5%), G12 (11.4%), G4 (6.4%), and G3 (4.3%). The G9 was dominant in this study and other regions of Iran; thus, this study was conducted to select an epitope from Rotavirus genotype G9 as a promising epitope candidate for future vaccine development. For this reason, several works including a complete sequence of VP7 G9, phylogenetic analysis, Prediction of Protein Structure, Physicochemical Properties of Protein and Epitope prediction were carried out.

The outcomes of this study revealed that the complete sequence of VP7 (G9) was comprised of 1062 nt with 326 amino acids (accession number MH824633). The selected epitope contained amino acid sequence of STLCLYYPTEASTQIGDTEWKN with the best score for T and B cells response.

Based on data of computational biology, the selected epitope can optimistically have considered as an epitope candidate for Rotavirus vaccine development.

Keywords: Computational biology; Rotavirus; Vaccines

INTRODUCTION

Rotavirus is a dsRNA virus belonging to the *Reoviridae* family and causes severe diarrhea in children below five years worldwide.[1] Every year, approximately 527000 children die from Rotavirus

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diarrhea. In fact, > 85% of these deaths take place in Africa and Asia.[2] Although Rotaviruses primarily infect enterocytes of the small intestine, it is found to be linked with the central nervous system (CNS) complications.[3]

Based on their antigenic specificity of VP7 and VP4, Rotaviruses have been classified into seven groups including A, B, C, D, E, F, and G. Group A Rotaviruses are the most common pathogenic of all and is only found in human. Group B and C Rotaviruses are zoonotic and some of these groups can also infect humans.[4] The Rotavirus genome comprises eleven segments of dsRNA, which encode twelve proteins. Among them, six are nonstructural protein (NSP1-NSP6) and six are structural proteins (VP1-VP4, VP6 and VP7).[5] Based on serological characteristics and sequence diversity of the outer capsid proteins, VP7 (glycosylated, G-type) and VP4 (protease-sensitive, P-type) viral classifications have been defined for Group A Rotaviruses (GARV).

Both VP7 and VP4 proteins induce neutralizing antibodies responses and are good targets for vaccine development.[6] So far, 27 G types and 37 P types have been identified in GARV.[7] High frequency of reassortment and interspecies transmission of Rotaviruses have been described resulting in the emergence of novel genotype.[8,9] Based on data provided by 21 studies, the prevalence of Rotavirus infection has been ranged around 15.3%-67.6% in some regions of Iran.[10] Rotavirus genotypes G1P [8], G2P [4], G4P [8], G9P (8) with human-dominant G9P [8] andG1P [8] genotype have been described in different places of Iran.[11,12] With regard to high mortality and morbidity rate due to Rotavirus infection and lack of specific treatment, the only preventive measure is the vaccination of children.

Until now, several live and attenuated vaccines were made but the main problem is a reversion of attenuated strain to a virulent form.[13] Rotashield (USA) licensed the first oral Rotavirus vaccine in 1998. Due to complications of intussusception, the vaccine was withdrawn.[14] Currently, two vaccinesRotaTeq™ (RV5; Merck & Co. Inc., USA) and Rotarix™ (RV1, GlaxoSmithKline, Belgium) are approved by the USA Food and Drug Administration (FDA) and used in many countries.[15] Due to the contamination of the Rotarix vaccine with viral porcine circovirus (PCV), the FDA suspended the vaccine in 2010. Later, PCV1 and PCV2 DNA were also detected in RotaTeq.[16] Since the PCVs would not threaten human life, both vaccines have been stated safe for use.[17]

Based on the unresolved safety concerns associated with live virus vaccines, new candidate vaccines have been produced, including inactivated Rotavirus, DNA vaccines, recombinant *E. coli* expressed Rotavirus proteins, etc.[18]

Regarding the high prevalence and human-dominant Rotavirus genotype G9 in Iran and other regions of the world,[8,19,20] the development of epitope vaccine is crucial. Thus, in sillico the B and T cells epitope prediction expression of VP7(G9) in *E.coli* was evaluated as a promising candidate for Rotavirus vaccine development.

MATERIALS AND METHODS

Ethics Statement

This project, with the registration number AJUMS - OG 94101, was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences Ahvaz, Iran. Consent was obtained from the child's parents.

Detection of Human Rotavirus

Briefly, 100 stool samples were collected from children with acute diarrhea under five years old who referred to the Aboozar Children's Hospital of Ahvaz city, Iran, during winter 2016. Following RNA extraction (Cinagen kit, Iran) and cDNA synthesize (Thermo Fisher Scientific, USA). Soon after the RNA extraction, the extracted dsRNA was converted to cDNA with a Thermo first strand cDNA Synthesis Kit (Thermo Fisher Scientific). The semi multiplex RT-PCR was carried out to detect and genotyping of human Rotavirus.[20] In addition, the entire Vp7 gene of the Human Rotavirus G9 genotype was obtained using specific primers Beg9/End9 (http://www.who.int/iris/handle/10665/70122).

Software Tools and Databases Used for the Prediction of Proteins Physicochemical Properties and Structure

Determination of physicochemical properties of protein exhibit critical impacts on protein activity, structure, and biological function. In the present study, the physicochemical properties of the amino acid sequence of the VP7 G9 [P8] in human Rotavirus were analyzed by the online tool Protparm.

Glycosylation Prediction of VP7 (G9)

Glycosylated proteins (glycoproteins) on the surface of the envelope viruses serve to identify and bind to receptor sites on the host's cellular membrane. In the present study, the N-linked glycoproteins, O-linked glycoproteins, and C-linked glycan of VP7 (G9) protein were analyzed; using a website (http://www.imtech.res.in/raghava/glycoep/submit.htm.

B-cell Epitope Prediction

The entire amino acid sequence of VP7 (G9) was aligned with one hundred amino acid sequences of human Rotavirus using the UniProtKB database (http://www.uniprot.org) and NCBI protein database (http://www.ncbi.nlm.nih.gov/protein) using MEGA 7.0. The highest score of B cell epitope prediction was selected by using Immune Epitope Database (IEDB) (http://tools.iedb.org/bcell/), Verification of predicted B cell epitope was analyzed by ABCpred Server (www.crdd.osdd.net/cgibin/abcpred) and Ellipro Server (www.tools.iedb.org/ellipro).

T-cell Epitope Prediction

To select epitope prediction by T cell, the entire amino acid sequences of VP7 (G9) were subjected to IEDB T cell epitope MHC2 tool (http://tools.immuneepitope.org/mhcii/), TepiTool (http://tools.iedb.org/tepitool/), and ProPred (http://crdd. osdd.net/raghava/propred/).

Antigenicity of the Selected Epitope

The antigenicity of B and T cells epitope prediction were further analyzed by Vaxijen 2.0 (threshold = 0.4), above the threshold 0.4 indicates antigenic (http://www.ddg-pharmfac.net/Vaxijen/Vaxijen/ Vaxijen.html).

Allergenicity of the Selected Epitope

The allergenicity of Band T cells epitopes prediction was done by the AlgPred server (http://crdd.osdd.net/raghava/algpred/index.html).

Secondary Structure and Three-dimensional Structure of Predicted B and T-Cell Epitope

The secondary and 3D structure of B and T cells epitope prediction of the VP7 (G9) were predicted by GORIV (http://cib.cf.ocha.ac.jp/bitool/GOR /GOR.php) and Swiss model server (https://swissmodel.expasy.org).

RESULTS

The results of our survey revealed that 30/100 (30%) specimens were positive for Rota RNA. The predominant G genotype was G9 (38.5%) followed by G2 (22.9%), G1 (16.5%), G12 (11.4%), G4 (6.4%), and G3 (4.3%).The complete sequence of VP7 G9 comprised 1062 nucleotide (nt), amino acids326 and deposited in the GenBank (MH824633)(Figure 1).

The Results of Physicochemical Properties of VP7 (G9) (Table 1).

The results of some of the outstanding physicochemical properties of the VP7 amino acid of G9 are presented in Table 1.

Results of Glycosylation

The results of N-linked glycosylation showed that can occur at positions 69 (serine) of VP7 (G9) was a potential glycosylated. The results of O-linked glycosylation showed at position amino acids [7,8(Threonine (T))], [70 (serine (S))], [78,80,87,108,200,202,210,214,245,272,276,277,278,281 (Threonine (T))] of VP7 (G9) were potential glycosylated. The results of C-linked glycosylation revealed that no potential glycosylated amino acid was observed in VP7 (G9) protein.

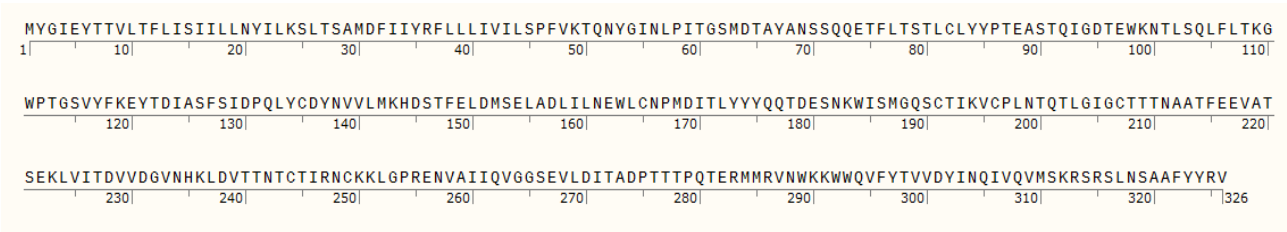

Figure1: Protein sequence of VP7 Genotype G9 isolated from Ahvaz, Iran

Table 1. Prediction of Proteins Physicochemical Properties and Structure of VP7

Virus Isolate	Description	Number of aminoacids	molecular weight(MW)	TheoreticalIsoelectric Point (PI)	Instability index(II)	Codon Adaptation Index (CAI)	Estimated half-life
Human Rota Virus Group A,G9	VP7(G9)	326 amino acid	37153.61 Dalton	4.81	35.19*	0.61	30 hours (mammalian reticulocytes, in vitro). >20 hours (yeast, in vivo). >10 hours (*Escherichia coli*, in vivo).

*Instability index: The instability index (II) is computed to be 35.19; this classifies the protein as stable.

B Cell Epitope Prediction

The results of the B cell epitope prediction sequence of VP7 (G9) showed the epitope with amino acid residues TEASTQIGDTEWKN (87-100aa) showed the highest antigenic score (1.5108) by Vaxijen (Version 2.0) (threshold=0.4) and selected as the desired B cell epitope.

T Cell Epitope Prediction–MHC-II Binding Prediction

The results of the T-cell MHC-II binding epitope prediction sequence of VP7 (G9) showed that the epitope with amino acid residues (STLCLYYPTEASTQI) (79-93aa) has found highest affinity binding to T-cell MHC-II (I,e., IEDB, TepiToll, and PROpred tools)**.** the highest antigenic score (0.5253) by Vaxijen (Version 2.0) (threshold=0.4).and selected as the desired T cell epitope

Finally, the selected amino acid (at position 87-93 residues, shown in bold,) in sequence **TEASTQI**GDTEWKN (at position 87-100 residues) and STLCLYYP**TEASTQI** (at position 79-93 residues) are predicted for B-cell and T-cell, respectively. Then, the final conserved epitopes of B-cell and T-cell showing an overlap were merged into a single peptide sequence of STLCLYYP**TEASTQI**GDTEWKN amino acids (22 aa) (at position 79-100) region of the VP7 (G9) region was selected

Result of Secondary and 3D Structures of B and T Cells Prediction Epitope

The results of the secondary structure of predicted B and T cells prediction epitope STLCLYYPTEASTQIGDTEWKN (22 aa) comprised of alpha-helix (h) 22.72%, beta sheets (e) 36.4% and random coil (c) 40.9 (Figure 2). The result of the tertiary (3D) structures of predicted B and T cells prediction epitope modeled by the Swiss model. The predicted epitope STLCLYYPTEASTQIGDTEWKN (22 aa) of surface glycoprotein VP7 (G) of humans is illustrated with Magenta color in Figure 3.

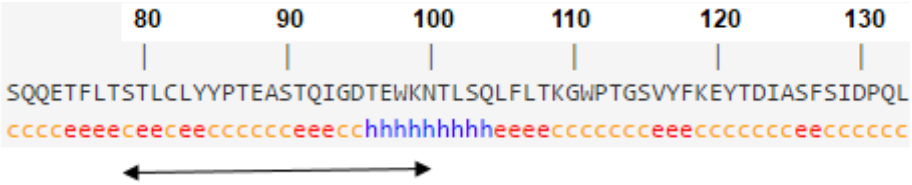

Figure 2. The results of the secondary structure of predicted B and T cells prediction epitope STLCLYYPTEASTQIGDTEWKN (22 aa) comprised of alpha-helix (h) 22.72%, beta sheets (e) 36.4% and random coil (c) 40.9%.

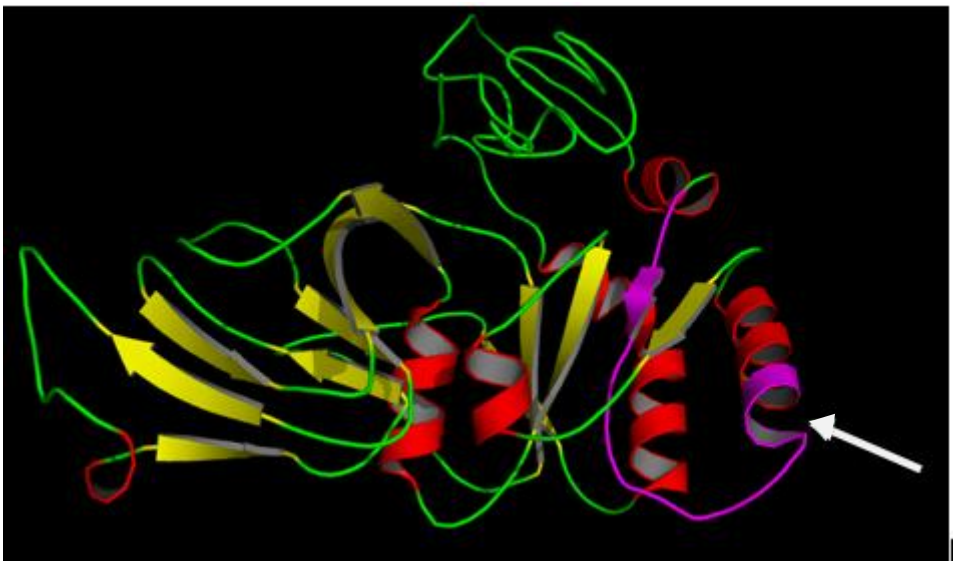

Figure 3: Predicted 3D Structure of complete sequence of VP7 strain G9 which indicated Helix strand (Red color), Sheet strand (Yellow color) and Loop regions (Green color). It should be noted Magenta color region (marked as while array) is representative of the STLCLYYPTEASTQIGDTEWKN sequence.

Antigenicity Results of the Predicted Epitope

The results consensus of B and T cells epitope prediction sequence of VP7 (G9) showed the epitope with amino acid residues STLCLYYPTEASTQIGDTEWKN (22aa) (79-100) showed the antigenic score (1.1014) by Vaxijen (Version 2.0) (threshold=0.4).

Evaluation of Allergenicity

The results allergenicity of the consensus sequence of the STLCLYYPTEASTQIGDTEWKN epitope showed non-allergenic properties (http://crdd.osdd.net/raghava/ algpred/index.html) and may predict a potent candidate epitope for the vaccine development.

DISCUSSION

Global molecular epidemiologic surveys have reported the genotypes G1P [8], G2P [4], G3P [8], G4P [8] and G9P [8] are the most common RVs cause of diarrhea in children worldwide.[8,19,22] Therefore, the goal of this study was to design outer capsid glycoprotein genotype VP7 (G9) epitope by in Silico B and T cells prediction, as a candidate epitope for the future vaccine development.

The results of physicochemical properties for stability index for VP7 (G9) was 35.19 and found stable while the instability index was predicted above 40. The result of expression prediction of sequences of VP7 (G9) in *E. coli* appears very poor and this showed a score of 0.61 (CAI) which was lower than the threshold. 0.8 by Codon Adaptation Index (https://www.genscript.com/tools/rare-codon-analysiswebsite). The previous studies have described the expression of VP7 in *E. coli* and eukaryotic cells have been failed.[23,24] The expression of VP7 (G9) of human Rotavirus is limited in yeast and plant. Recently, the expression of VP7 G9 failed in N. benthamiana.[25]

The epitope (TEASTQIGDTEWKN) (at position 87-100 aa) of VP7 (G9) was designed for B-cell epitope prediction and showed the highest antigenic score (1.5108) by Vaxijen (Version2.0). The selected epitope was located in the hypervariable region of VP7(G9). The epitope (STLCLYYPTEASTQI) (at position 79-93 aa) of the VP7 (G9) gene was designed by (I,e., IEDb, TepiToll, and PROpred tools) and showed the highest affinity for T-cell MHC-II binding epitope, with the antigenic score (0.5253) by Vaxijen (Version 2.0).

Finally, selected amino acid residues (at position 87-93, shown in bold,) in sequence **TEASTQI**GDTEWKN (at position 87-100aa) and STLCLYYP**TEASTQI** (at position 79-93 aa) of VP7 region are the B-cell and T-cell prediction epitopes

respectively. Therefore, the final conserved epitopes of B-cell and T-cell showing an overlap were merged into a single peptide amino acid sequence of STLCLYYP**TEASTQI**GDTEWKN were selected which showed antigenic score (1.1014) by Vaxijen (Version 2.0) and found to be non-allergenic properties by AlgPred tool (ARPs method). The selected epitope for B and T cells epitope showed no N-link Glycosylated Sites, only O-linked Glycosylated Sites predicted at positions of 80 and 87the VP7 (G9) region. Although the previous study showed that glycosylation ofVP7 was not required for B-cell activation by Rotavirus.[26] It was found that the appropriate epitopes of the VP7 region can induce B cell activation. Hart et al have demonstrated three epitopes in variable regions (VR) of VP7(G9) region including (aa 87–101), VR7 (142–152) and VR8 (aa208–221) are the major neutralizing epitopes.[27] However, in our survey, the selected epitope (87-100aa) was predicted as a major epitope for B cell response.

The selected STLCLYYPTEASTQIGDTEWKN epitope VP7 G9 (magenta color in Fig 3) was predicted to induce Band T cell activation. Furthermore, based on the results of a protein blast database, the selected epitope did not show any homology to the human peptide (https://blast.ncbi.nlm.nih.gov). Based on the aforementioned data The selected STLCLYYPTEASTQIGDTEWKN epitope may predict as a promising candidate for the development of the Rotavirus vaccine.

The presently licensed Rotavirus vaccines are not cost-effective for less and the least developed countries. Recently, considerations were focused on economically affordable use of immune epitopes in the form of multivalent dosages as a promising candidate subunit vaccine development against Rotavirus infection. Finally, the design of potent epitope which induces B cell and T cell activations is an important consideration for vaccine development.[28,29]

In this study, the physicochemical properties of VP7 G9 gene proved to be stable. The primary structure of VP7 (G9) comprised of 326 amino acids. The secondary structure of the selected epitope showed helix, sheet, and coil region, which contained the disordered region. The designed STLCLYYPTEASTQIGDTEWKN epitope from outer glycoprotein of the Rotavirus VP7 G9 region showed a highly conserved, immunogenic and induce B and T cells activations and with non-allergenic features. Our conclusion is that the selected epitope retrieved from the VP7 G9 region of Rotavirus may predict a potent candidate epitope for vaccine development.

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