Estimation Efficacy of Rotarix Vaccine of Zoonotic Rotavirus by Insilco Production Epitopes

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Abstract

The aimed of this study was to detect the efficacy of rotavirus vaccine (Rtarix vaccine) by estimation of its potential antigenicity to induce immunity through insilco production with bioinformatics tools therefore vp7 gene (glycoprotein) was chosen to analyses its antigenicity because it is one of Rotarix vaccine component, fifty outer capsid of *vp7* gene proteins from human Rotavirus A of different strains from different countries with desired amino acids by Immunobioinformatic tools these desired amino acids selected as conservancy region to describe an epitope based peptide vaccine design against Rotavirus, using a combination of T-cell and B-cell epitope predictions. To perform this, sequences of Rotavirus VP7 proteins were retrieved from the NCBI database (genbank) and subjected to different bioinformatics tools to predict most immunogenic T-cell and B-cell epitopes. From the identified epitopes, the sequence NPMDITLYYY of VP7 was identified as the most potential epitopes based on their antigenicity, conservancy and interaction with major histocompatibility class I and class II (MHC-I and MHC-II) alleles. Moreover, the antigenicity score (2.7150) for vp7 . Combined population coverage for our identified epitopes was found 92.09% at a higher percent and 41.26% for United States and South west Asian (Iraq) respectively. All these results suggest that, the epitopes identified in this study could be considered as less potential for vaccine candidate for the strains of Rotavirus circulating in Iraq therefor further studies are needed for improvement of Rotarix vaccine and the update vaccine should contain variant strains from variant species such as animal species because it is a zoonotic virus and have ability to reassortment between human and animal.

Keywords: bioinformatics tools, epitopes, vp7 protein

تقيم كفاءه لقاح روتاركس لفايروس الروتا الانتقالي عن طريق استنباط الابتوبات في الكومبيوتر.

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الخلاصه

هدف هذه الدراسة هو فحص كفاءه لقاح فايروس الروتا (لقاح روتاركس) من خلال حساب فعاليته المضادة لحث المناعة باستخدام برامج المعلوماتية الحيوية لذلك تم اختيار جين VP7 (كلايكوبرتين) لتحليل قابليته المناعية كونه احد مكونات لقاح روتاركس, لإنجاز هذ العمل تم اختيار 50 بروتين تابع للجين الخارجي VP7 من خلال موقع بنك الجينات مع

تحديد الاحماض الأمينة المطلوبة بواسطه برامج المعلوماتية الحيوية المناعية, الاحماض الأمينية التي يتم اختيارها يكون على اساس التشابه بين هذه البروتينات لتحديد الابتوبات التي تدخل في تصميم اللقاح ضد فايروس الروتا لخلايا T-Cell, B-cell NPMDITLYYY , هذا التسلسل للأحماض الأمينية يعتبر الأقوى من حيث التحفيز المناعي وقابليته على الارتباط مع معقد التوافق النسيجي من النوع الاول والنوع الثاني وهذه الابتوبات سجلت تحفيز جيني بمقدار (2.7150) في حين قابليه هذه الابتوبات على التغطية المناعية للأفراد كانت (92.09%) في امريكا كأعلى نسبه للتغطية اما جنوب غرب اسيا الذي يشمل العراق كانت التغطية المناعية (41.26 %) , من خلال هذه النسب اثبتت هذه الدراسة انه الابتوبات المشخصة في اللقاح هي ذات قوه ضعيفة من حيث التغطية المناعية للأفراد ولا يمكن ان تعطي مناعة كافيه في العراق لذلك نحتاج الى دراسات اضافيه لتطوير لقاح الروتاركس و يجب العمل على تحديث هذا اللقاح بإدخال عتر مختلفة من مضائف مختلفة مثل الحيوان كون فايروس الروتا هوه فايروس انتقالي من الانسان والحيوان وله القابلية على اعاده تركيب جيناته.

1. Introduction

Rotavirus A considered as one of the important members of zoonotic diarrheal disease in human and animal specially in young children and calves therefor this virus sharing many genetically features between those two hosts (1).Rotavirus (RV) is one of the members of *Reoviridae* family which is contain double stranded RNA (dsRNA) with different species classified from (RVA to RVH) (2).While Desselberger (2014) (3) detected non-structural proteins about NSP1 to NSP6 with eleven dsRNA segments which code structural protein there are (VP7,VP6,VP4-VP1) and confirmed the activity of VP7 gene for attachment and entrance of Ratavirus. On the other hand (4) showed the vital role of VP6 trimers to assist VP7 trimers for its attachment in outer cell surface whereas (5) revealed that attachment partners on the host cell surface, containing by sialoglycans and histo-blood group antigens (HBGAs) In fact, alterations in rotavirus epidemiology among populations had been affected by genetic differences in HBGA expression (6).The function of HBGAs in the related effect on vaccine efficacy and susceptibility to rotavirus vaccine strains are important developing areas of research attended with public health (7). Rotavirus attached and neutralized their antigen through the structural protein in outercapsid which are vp4 and vp7 genes (8). TM (Wyeth-Lederle Vaccines) was pullout according to its side effect with intussusception in 1999. Then in 2006 another vaccine was improved with prevalence of recombination human –bovine and finally RotatixTM (9). Recently WHO viewed 101 countries implemented rotavirus vaccine at the end of 2018 with international coverage about 35% for ratavirus infection as it is a common source of infection with severe diarrheal in children global (10). The extra intestinal influence of the rotavirus vaccine, which is called the “rotavolution”, has recently become a growing field of debate and research (11).

The infection of rotavirus in animal observed mainly in youngest during weaning period therefor the theory of passive immunization can induced by antibodies from mothers which transported through placenta or released with colostrum in early days after birth to stimulate active immunity (12). The pregnant animals to raise of antibody level should be immunized with multivalent vaccines having the important antigens causing an enteric infection specially in late stage of pregnancy (1). In cows colestrum with increase immunity very important in calves to give protection (13) also in infant mice have a high immunity (14). Rotaviruses detected in animals, even those similar to human viruses or having cross transmission abilities, rarely infect humans in nature and their zoonotic significance is yet to be discovered and the pathological lesions detected in calves are mucosal congestion in

small intestine and find of catarrhal exudate in the lumen. The affected parts of the intestine show necrosis and desquamation of villous epithelium, shortening of villi and mononuclear cellular infiltration in sub-mucosa (15).

Utilization vaccination programs as an apparatus in protection wide range diseases has been considered the main steps in the fight against various infectious diseases such as viral disease one of them rotavirus (16). At present, epitope-based vaccine project using in silico methods is a very favorable method of vaccine progress due to its period and price effectiveness. Some new studies display that, epitope established vaccination plans can professionally provoke the immune response against different pathogen (17).

Material and methods

Retrieving *vp7* Protein Sequences of Zoonotic Rotavirus A

Materials

The fifty outer capsid of *vp7* gene proteins of human rotavirus A of different strains from different countries specifically United States, , Brazil, Russia, Thailand, Italy, British, Turkey, Pakistan, Iran, Argentina, India, Kenya and local Iraq's strains were retrieved from NCBI (18).The highly restored and usually appeared sequences applied with FASTA format. To illustration by immunoinformatics tools. The restored sequences of VP7 protein were accumulated in distinct file for aligning by MEGA 7.0.18 software (19). Multiple aligning of sequences was done through ClustalW algorithm then phylogenetic tree draw the correlations between these multiple sequences by Maximum Likelihood Method.

Methods

Immune Epitope prediction

Detection of highest antigenic conserved Region for vp7 protein sequences was detected by the VaxiJenv2.0 server in which the default parameters was used for the identification(20).

Identification of Antigenicity

To identify cytotoxic T lymphocyte (CTL) epitopes from the most antigenic conserved region aserverentitledNetCTL1.2 was used which is based on neural network architecture. To evaluate the optimal score a combination network should done between major histocompatibility complex class -I (MHC-I),transport proficiency, proteosomal cleavage expectation and transporter of antigenic peptide (TAP).the score value 0.75 was selected as a good threshold sensible for sensitivity and specificity. In vivo the prediction of epitopes with utilizing twelve human leukocyte antigen (B62, B58, B44, B39, B27, B8, B7, A26, A24, A3, A2 and A1) these epitopes were dependent in this attitude (21) .

Immune Epitope Database

Prediction of T-cell Epitope

Conservancy analysis apparatus was don on best selected epitopes (22) . The epitopes having about 80% conservancy were selected for existing their half maximal inhibitory concentration (IC50) rate followed by attaching to human leukocyte antigen (HLA) by stabilized matrix method (SMM) (23).In this study, the development of vaccine based on highest conservancy analysis which were selected to exist their corresponding HLA attaching

at IC50 value <500nM. MHC-II molecules cover epitopes the best elected epitopes also detected by SMM (24).

B cell epitope prediction.

B-cell epitope prediction was detected through Imuno Epitope Database (IEDB) tool after higher vaxijen value was detected for vp7 protein sequences (21). potential antigenicity was secerned through Kolaskar and Tongaonkar antigenicity tool (25).

Analyzing Epitope Conservancy.

Imuno epitope database analysis resource was used to analyze the epitope conservancy for each individual predicted epitopes. The given protein sequences were searched in this web based tool for identities to calculate the conservancy level of all predicted epitopes (25). This tool estimates average number of HLA to epitope incorporation approved by the population of different environmental distributions.

Population Coverage Prediction.

IEDB population coverage calculation tool was used for the election of population coverage of every individual epitope. To predict the population coverage for the analogous epitope the allelic frequency of the interacting HLA alleles were hired (25).

3D structured Structure for epitope prediction

PyMol server, which is a denovo method planned to predict peptide structures from amino acid sequences was hired to build the three dimensional (3D) structure of the highest conserved T-cell epitope (26).

3.Results

Analysis of Local Rotavirus protein Sequences

A fifty outer capsid vp7 protein of human RotavirusA from different countries with Rotarix strain that obtained by NCBI database and local Iraq's strains were selected and analyzed to investigate their relationships. The results propose that they are very similar in distance by performing multiple sequence alignment and phylogenetic trees. In order to explore the genetic diversity of Local Iraqi Vp7 sequence protein in pink color were most homologous with Russian strains in blue color while red color indicated to Rotarix strain shows highly related with Argentine, Thailand and Pakistan then the purple color indicated the less related strains with Iraqi strain were from Russia , USA, Brazil and Venezuela figure 1**.**

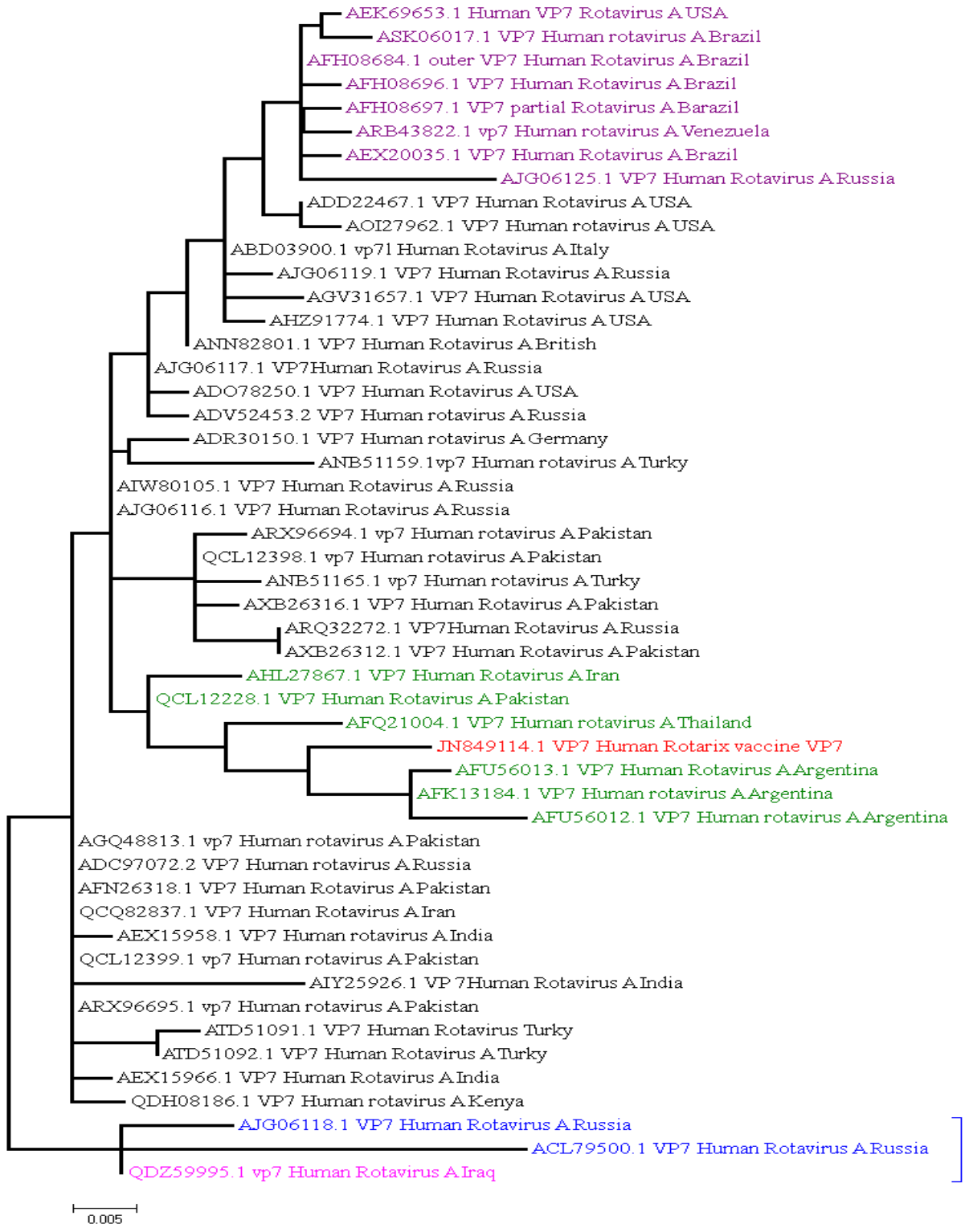


Figure (1): Rotavirus A *vp7* protein region phylogeny for both local and international sequences molecular phylogenetic analysis was done by maximum likelihood method. The boot strap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the sequences analyzed pink color =local Iraq, blue color =closely related with Iraq strain, red color =Rotarix vaccine, green color= close related with Rotarix vaccine, purple color=less related from Iraq sequence and the rest samples are international sequences .

Imunobioinformatic analysis of Rotaviral predicted protein.

The 50 outer capsid VP7 protein of Human rotavirus A from Iraq and diverse countries were achieved and analyzed for their estimation correlation. Six conserved regions of more than 10 amino acids were recognized from the multiple sequence alignment of 50 saved proteins of VP7 proteins indicate the ability of protein sequence to be probable Antigen as a protective antigen in vitro design and the highest antigenicity score was obtained by sequence "LGIGCQTTNVDSFEMVAENE" in compared with scores of different sequences of multiple alignment according vaxigen tool, figure 2,3 and table 1.

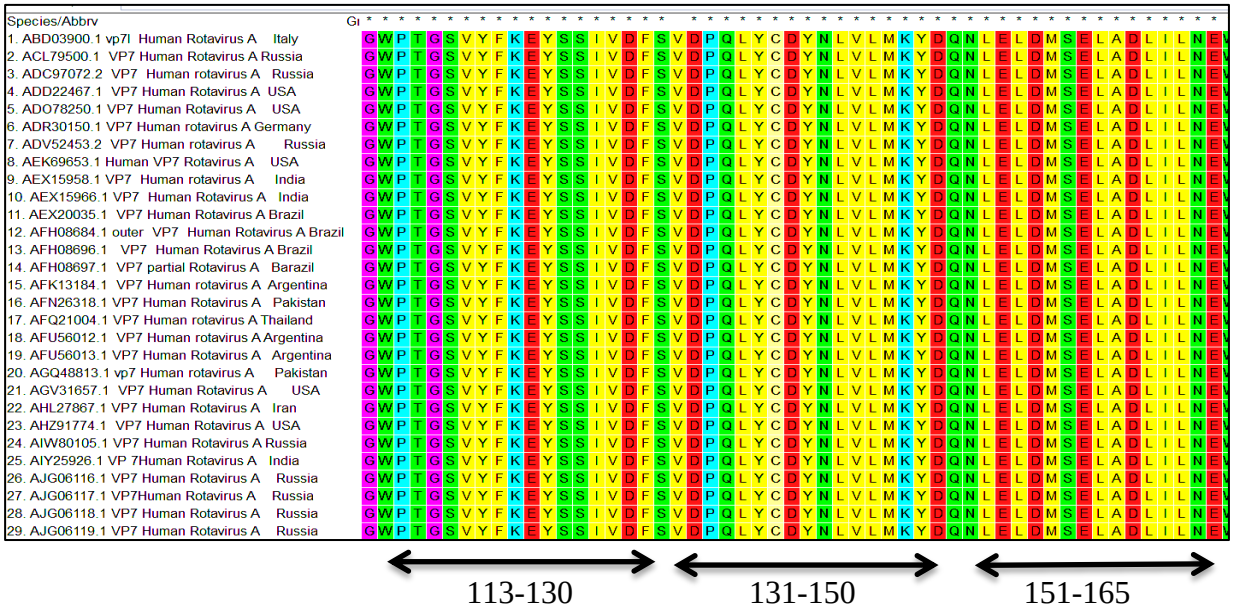


Figure (2): Multiple sequence alignment of vp7 proteins showed three conserved regions of more than10 amino acids from different strains of Rotavirus A.

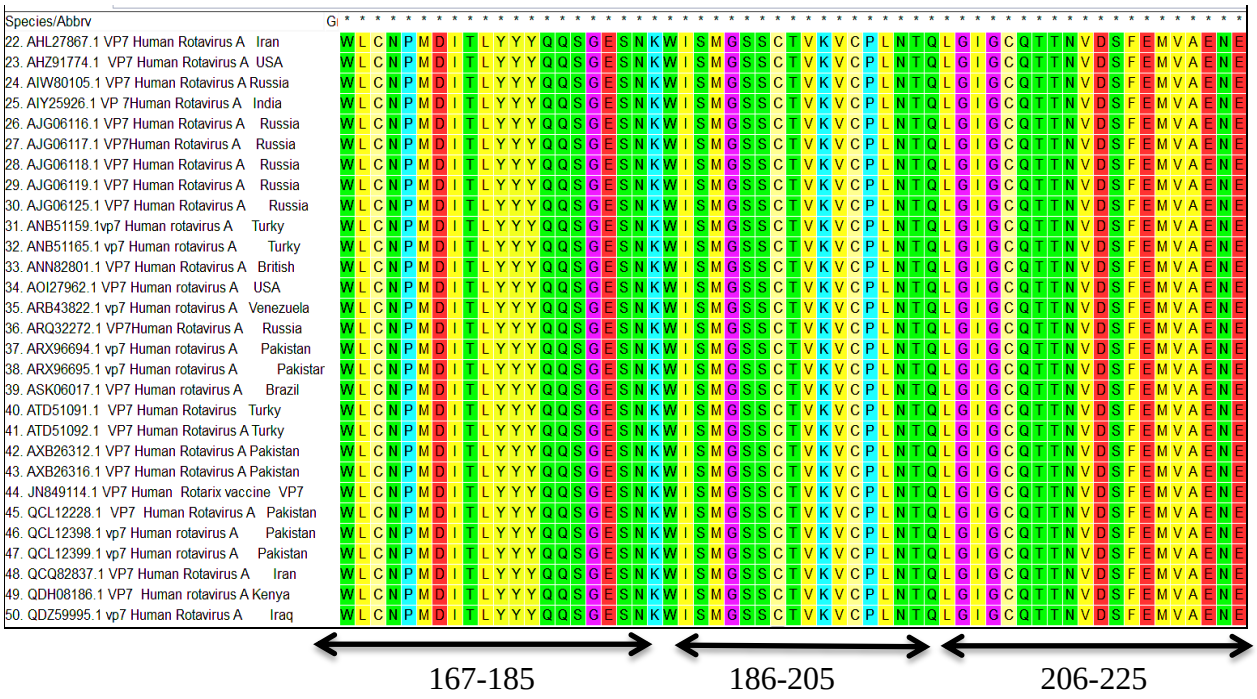


Figure (3): Multiple alignment of vp7 proteins showed another three conserved regions of more than10 amino acids from different strains of Rotavirus A.

Table (1): Conserved region of VP7 from multiple sequence alignment and their antigenic score.

Conserved region no.	Amino acid sequence	Position of conserved regions amino acids	Vaxijen score threshold (0.4)
1	GWPTGSVYFKEYSSIVDF	113-130	0.7306
2	SVDPQLYCDYNLVLMKYDQN	131-150	0.7836
3	LELDMSELADLILNE	151-165	0.6186
4	WLCNPMDITLYYYQQSGESN	166-185	0.6910
5	KWISMGSSCTVKVCPLNTQ	186-205	0.7072
6	LGIGCQTTNVDSFEMVAENE	206-225	1.0783

Prediction of TCell Epitope of rotavirus proteins

Aminoacids"TQINDGEWKDSLSQMFLTKGWPTGSVYFKEYSSIVDFSVDPQLYCDYNL VLMKYDQNLELDMSELADLILNEWLCNPMDITLYYYQQSESNKWISMGSSCTVKVC PLNTQTLGIGCQTTNVDSFEMVAENE" of VP7 proteins were selected for analyzed epitope detection by NetCTL web tool at threshold value 0.75 . The NetCTL score values combination of transporter associated with antigen processing (TAP) proteasomal cleavage/MHC-I united predictor for vp7 proteins, table 2.

Table (2): The predicted epitopes vp7 protein on the basis of their overall score of Tcell epitope predicted by the NetCTL server.

Number	Epitopes	length	Overall score (nm)
1	VYFKEYSSI	9	1.0270
2	VLMKYDQNL	9	1.1490
3	CQTTNVDSF	9	2.5030
4	PMDITLYYY	9	2.5310
5	GWPTGSVYF	9	2.6880
10	NPMDITLYY	9	2.7150
11	DYNLVLMKY	9	2.7720
12	KGWPTGSVY	9	2.9470

MHCI Epitope Prediction for *vp7*gene

The elected T-cell epitopes were exposed to MHC-I binding prediction. Then stabilized matrix method (SMM) was utilized to predict IC50 for the epitopes with MHC-I in binding condition. Epitopes with IC50 < 500nM displays moderate affinity with MHC-I and those produced comparatively higher affinity (IC50 < 500nM) were selected for next analysis. The results confirmed by another web T-Cell. In view of fact netCtle show regarding analysis of vp7 gen in comparison of multiple aminoacids showed interaction with HLA in vitro. Epitope Prediction revealed that among the VP7 epitopes NPMDITLYYY suggested to higher interact

with HLA by netCtle then category of T cell epitope prediction to *Vp7* gen that processed in MHC- I ligand, NPMDITLYYY was detected as most suitable epitopes prediction which interact with HLA-A*01:01, while PMDITLYYY interact with HLA-C*04:01 also another two epitope interact with more than one allele illustrate the first one ELADLILNEW interact with HLA-B*58:01 and HLA-B*44:02, the second epitope LCNPMDITLY interact with HLA-A*01:01 and HLA-B*39:01 table 3.

Table (3): the best predicted Tcell epitopes of vp7 protein which interact with more diverse MHC-I alleles .

N0	Epitope	SMM IC50	MHC-I allele	the score of percentile rank	Epitope conservancy rate (%)
1	NPMDITLYYY	517.37	HLA-A*01:01	0.4	40.00%
2	ELADLILNEW	269.31	HLA-B*58:01	0.5	40.00%
3	ISMGSSCTVK	67.80	HLA-A*03:01	0.7	30.00%
4	GSSCTVKVCP	335.16	HLA-B*58:01	0.8	30.00%
5	YYYQQSGESN	351.33	HLA-A*24:02	0.8	40.00%
6	LCNPMDITLY	868.56	HLA-A*01:01	0.8	40.00%
7	VCPLNTQTL	899.95	HLA-E*01:01	0.8	55.56%
8	DMSELADLIL	383.93	HLA-B*58:01	1	40.00%
9	SELADLILNE	454.87	HLA-B*44:02	1.3	40.00%
10	CPLNTQTLGI	312.48	HLA-B*07:02	1.4	50.00%
11	ILNEWLCNPM	23.13	HLA-B*15:01	1.7	40.00%
12	LCNPMDITLY	24.28	HLA-B*15:01	1.8	40.00%
13	MSELADLIL	489.10	HLA-B*39:01	2.1	33.33%
14	PMDITLYYY	2479.82	HLA-C*04:01	2.1	44.44%
15	ELADLILNEW	747.98	HLA-B*44:02	2.2	40.00%

MHC- II Epitope Prediction for rotavirus vp7

The latest versions of the prediction epitopes in T-cell with low Inhibitory Concentration (IC50) are listed in table 4.Which eluted from MHC molecules that implemented for the prediction of MHC-II molecules that cover the best candidate epitope from the selected conserved region of vp7 proteins observed across MHC-II immune responses across all tested regions.

Table (4): the best potential vp7 epitopes which interact with more diverse MHC-II alleles

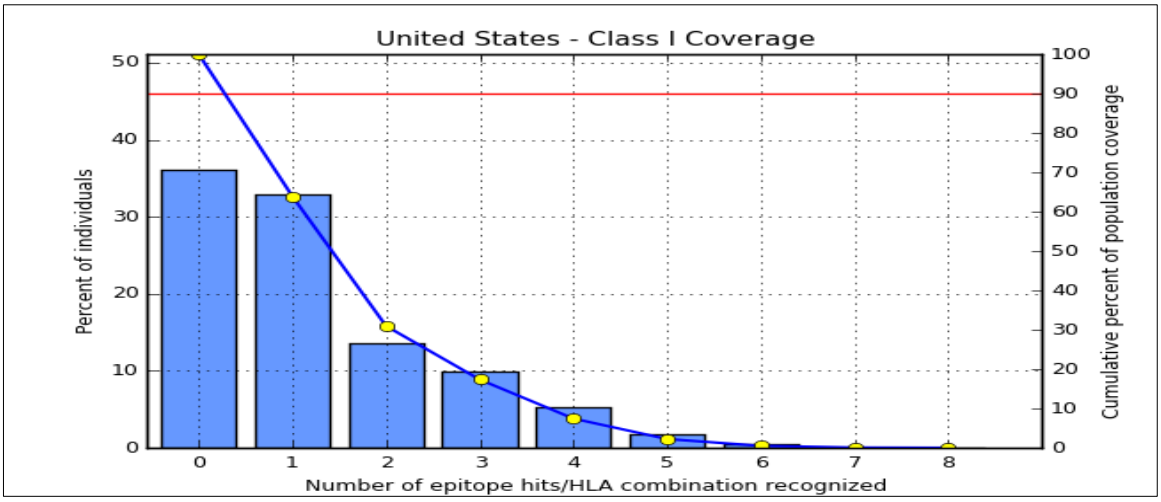
N0	Epitope	SMM IC50	Interacting MHC-I allele with an affinity of <500nM	Score of percentile rank	Epitope conservancy rate (%)
1	SNKWISMGSSCTVKV	18.00	HLA-DRB1*01:01	2.60	33.33%
2	NKWISMGSSCTVKVC	18.00	HLA-DRB1*01:05	2.60	33.33%
3	ESNKWISMGSSCTVK	19.00	HLA-DRB1*01:05	2.90	33.33%
4	GESNKWISMGSSCTV	20.00	HLA-DRB1*01:05	3.10	33.33%
5	SGESNKWISMGSSCT	22.00	HLA-DRB1*01:05	3.60	33.33%
6	DYNLVLMKYDQNLEL	41.00	HLA-DRB1*01:05	1.50	33.33%
7	YNLVLMKYDQNLELD	41.00	HLA-DRB1*01:03	1.50	33.33%

Population Coverage Prediction

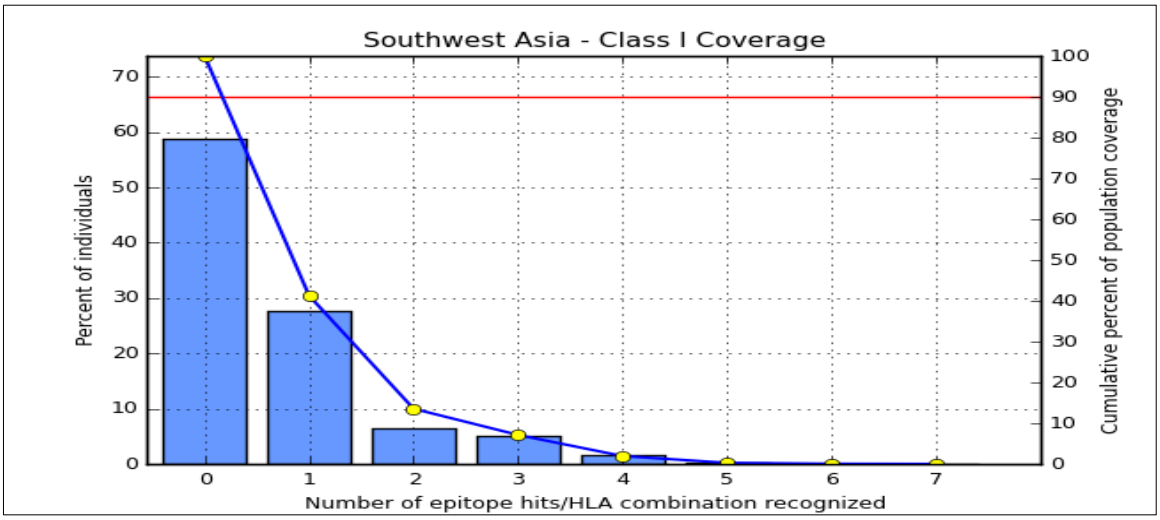
The Human Leukocyte Antigen (HLA) dissemination analysis was undertaken to choose the most promiscuous T-cell epitopes using the population coverage tool of Immune Epitope Database (IEDB). These epitopes have been detected on the basis of their binding ability with maximum number of HLA alleles along with highest population coverage rate values for all the geographical areas studied. The comparative population coverage analysis of moderately immunogenic and high immunogenic peptides suggests that the former may activate T-cell response in a fairly large proportion of people in most geographical areas, thus demonstrating their potential for development of epitope-based vaccine the result arranged according to population coverage percentage was regarded for vp7 epitopes NPMDITLYYY. Combined world population coverage in vp7 epitopes results according MHC-I interaction of highest population coverage in world region was found in 0 92.09% at average hit 1.78 and Southwest Asia ethnic region was calculated 41.26% at average hit 0.81. figure 4, table5.

Table (5): Combined population coverage for VP7 epitopes.

Area	Coverage	Average hit	pc90
United States	92.09%	1.78	1.05
Germany	78.75%	1.5	0.47
Thailand	76.84%	1.32	0.43
Russia	70.27%	1.24	0.34
Italy	64.58%	1.32	0.28
Brazil	50.0%	0.81	0.2
Venezuela	48.72%	0.55	0.19
South Asia	46.69%	0.85	0.19
India	45.78%	0.81	0.18
Southwest Asia(Iraq)	41.26%	0.64	0.17
Pakistan	39.28%	0.57	0.16
South Africa	39.25%	0.76	0.16
Iran	28.79%	0.51	0.14
Argentina	25.47%	0.53	0.13



(A) number of vp7 epitope hits/HLA combination recognized



(B) number of vp4 epitope hits/HLA combination recognized

Figure (4): Vp7 epitope combined population coverage (A) the world and (B) Southwest Asian ethnic region based on MHC restriction data using the Immune Epitope Database analysis resource.

Prediction of B-Cell Epitopes

Kolaskar & Tongaonkar Antigenicity Results

The *vp7* potential protein epitopes sequences have been identified by linear B cell epitopes tool. That Kolaskar and Tongaonkar's used as B cell antigenicity method for vp7 protein, conserved region were employed in table 6 showed ITLYYYQ aminoacides between 80-86 fragment estimated affinity regions for B-Cell Epitopes, figure 5 showed higher four antigenicity regions then antigenicity scores were documented in table 7.

Table (6): Predicted vp7 peptides

No	Start	End	Peptide	Aminoacid number
1	24	29	GSVYFK	6
2	32	53	SSIVDFSVDPQLYCDYNLVLMK	22
3	80	86	ITLYYYQ	7
4	100	109	SCTVKVCPLN	10

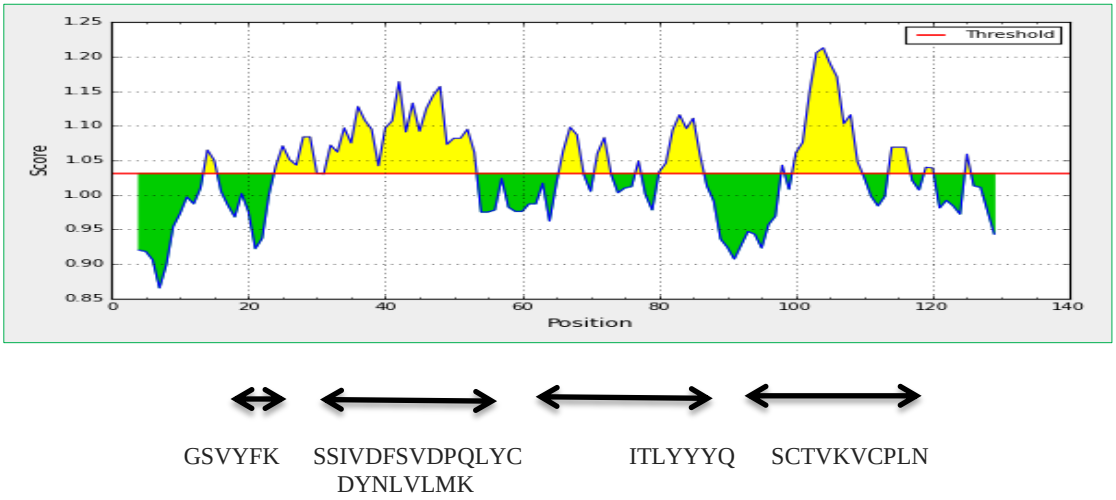


Figure (5): Kolaskar & Tongaonkar Antigenicity Results for vp7 protein.

Table 7: Predicted vp7 residues scores

Position	Residue	Start	End	Peptide	Score
50	V	47	53	YNLVLMK	1.082
51	L	48	54	NLVLMKY	1.082
101	C	98	104	GSSCTVK	1.076
35	V	32	38	SSIVDFS	1.075
49	L	46	52	DYNLVLM	1.073
32	S	29	35	KEYSSIV	1.072
25	S	22	28	PTGSVYF	1.071
114	G	111	117	QTLGIGC	1.069
115	I	112	118	TLGIGCQ	1.069
116	G	113	119	LGIGCQT	1.069
14	Q	11	17	SLSQMFL	1.065
66	A	63	69	SELADLI	1.064
33	S	30	36	EYSSIVD	1.062
53	K	50	56	VLMKYDQ	1.062
100	S	97	103	MGSSCTV	1.061
71	N	68	74	LILNEWL	1.06
125	F	122	128	VDSFEMV	1.059
86	Q	83	89	YYYQQSG	1.057
26	V	23	29	TGSVYFK	1.051
50	V	47	53	YNLVLMK	1.082
51	L	48	54	NLVLMKY	1.082
101	C	98	104	GSSCTVK	1.076
35	V	32	38	SSIVDFS	1.075

49	L	46	52	DYNLVLM	1.073
32	S	29	35	KEYSSIV	1.072
25	S	22	28	PTGSVYF	1.071
114	G	111	117	QTLGIGC	1.069
115	I	112	118	TLGIGCQ	1.069
116	G	113	119	LGIGCQT	1.069
14	Q	11	17	SLSQMFL	1.065
66	A	63	69	SELADLI	1.064
33	S	30	36	EYSSIVD	1.062
53	K	50	56	VLMKYDQ	1.062
100	S	97	103	MGSSCTV	1.061
71	N	68	74	LILNEWL	1.06
125	F	122	128	VDSFEMV	1.059
26	V	23	29	TGSVYFK	1.051

Predicted epitopes

From a serial approach for epitope prediction the best epitopes were selected "NPMDITLYYY" according to their potency of antigenicity and population coverage . The 3D structure of VP7 protein was predicted by using pymol server displayed in figure 6 . The residues in most favored regions in red colored

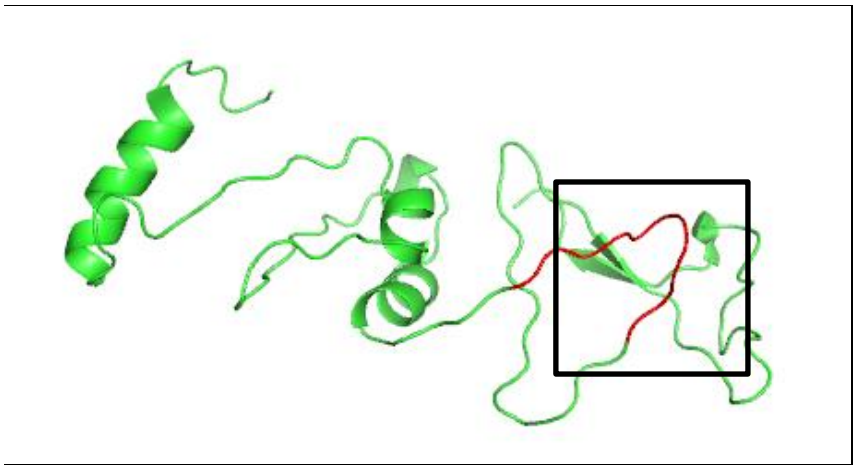


Figure (6): Selected region for T-cell and B-cell epitope from built model. red region indicates the Rotarix vaccine epitope region of 3-D structure for vp7 protein

4. Discussion

Analysis of Local Rotavirus protein Sequences

In the current study, we compared the VP7 protein and RotavirusA vaccine (Rotarix vaccine) which was introduced in Iraq Since 2016 with those of the presently flowing Rotavirus antigens in Iraq and international. In particular, comparing by phylogenetic tree between local and international *vp4* protein assumed there is correlated relationship was observed between Iraqi strain and recent vp7 strains reported from Turkey , India, Pakistan, Russia and USA, Local *Vp7* protein was most homologous with Russian strains whether Rotarix vaccine strains shows highly related with Argentine, Thailand and Pakistan figure1 this relation pours into the goal of the present study and may draw a picture of important

amino acids that reveled in vaccine preparation which considered as an epitope for immunity stimulation and continuous observing for diversity of Rotavirus genotypes is necessarily in the future with update epitopes prediction. agree with (22) revealed the important of conclude the changeability of epitopes during a given usual of protein sequences by developing a new tool to support in the assortment of epitopes with the favorite grade of conservation.

Imunoinformatic of Rotavirus epitope Prediction with vaxigen Analysis Conserved sequences

The first result with epitope prediction indicate the ability of vp7 protein sequences to be probable antigen versus probable non antigen as a protective antigen in vitro design according to higher antigenicity score which was obtained when the activity of a sequence may be encoded in accomplished and ambiguous way that not adjustable to direct detected by sequence association. According bioinformatics tool with high antigenicity "LGIGCQTTNVDSFEMVAENE" considered highest conserved region figure 2, 3 Table 1 for vp7 after multiple alignment for vp7 proteins of Rotavirus A strains from different regions. Was chosen for collected of amino acid residues that are usually current in diverse protein sections, nevertheless which are carried all by protein compacting these results compatible with (20) who revealed the important of alignment by Bioinformatics tool depending on physiochemical activities of protein then these set was achieved to induce the optimal antigenicity of complete protein resulting but these aminoacids not dependent as abest epitope because further analysis should be done to confirm this antigenicity.

Prediction of T-Cell Epitope of rotavirus proteins

Conversely, the segment of protein sequences which comprise the epitope measured below a indicated level of individuality reveals the degree of changeability or individuality of the derivatives epitope depending on it acceptability for T-cell "TQINDGEWKDSLSQMFLTKGWPTGSVYFKEYSSIVDFSVDPQLYCDYNLVLMKYD QNLELDMSELADLILNEWLCNPMDITLYYYQQSESNKWISMGSSCTVKVCPLNTQTL GIGCQTTNVDSFEMVAENE" of VP7 protein was selected termed as the highest NetCTL score (1.0270) for vp7 protein this epitopes in table 2.

Furthermore, these districts usually characterize respectable objectives for the improvement of epitope depending in vaccines, like these epitopes can be estimated to be extant regardless of disease period, or specific strain of the pathogen. Moreover, the similar residues are usually considered as a best conserved region through changed associated species, this result agree with (22) who conducted the Immune Epitope Database and Analysis Resources (IEDB) tool can be utilized for stalking mutation of epitopes throughout disease development.

MHC-I and MHC- II Epitope Prediction for Rotavirus *vp7*protein

Epitope is probable deliver the protection approximately in coordination of all previous strains depending on affinity between human leukocyte antigen (HLA) and epitopes is expected as the effectiveness of vaccines against different strains this view agree with (23) explained about the important of resources Immune Epitope Database (IEDB) for helping in the selection of epitopes and industrialized an epitope by approach which explain analysis in detail of conservancy regions.

The present study celebrated that more interactions with Major Histocompatibility class1 (MHC-1) molecules according lesser Score of percentile rank were perceived when higher concentrations(>500nM) were holed. To approve the necessary attraction, epitope was binding to both MHC-I and MHC-II allele and take the more frequently interactions with MHC-1 and molecules MHC-II according to present study demonstrate the NPMDITLYYY epitopes interact with HLA-A*01:01and PMDITLYYY interact with HLA-C*04:01 these epitopes were chosen as best epitopes according many consecrations the important of them were lower score of percentile rank and variation of HLA which interact with them table 3.
The accuracy of MHC-I binding prediction discovered that this approach can handle even small virus in tens of thousands peptides fragments these residue seemed diverse default value according to its coupling with MHC and assortment of a perfect set of peptides are combined to produce the all binding intended for given application independent on proteasomal cleavage transport associated protein. And is an computerized technique that can automatically gather the HLA with targets of epitopes, assumed abundant datasets of T-cell reactions in HLA typed topics (27). While in current study for MHC-II fragments epitopes SNKWISMGSSCTVKV are more affinity to MHC-II allele specially HLA-DRB1*01:01 for vp7 were selected as their lower Inhibitory Concentration (IC50) about (18.00) table 4. The present study publicized the important antigen offering by (MHC) to generate potent peptides for adaptive immunity in vaccine design compatible with (28) reported that the peptides need to be created from proteins (MHC) are prearranged by profuse genes which have an important effect for peptide interaction according to its polymorphism also compatible with (29) who revealed the epitope action and vaccine project is progressively depending on imunoinformatics analysis apparatuses and arrival to chosen data pertinent to immune activities and definite pathogens by combined class I and class II Major Histo Compatible prediction tools, groups of HLA alleles have been detected deliver over 95% worldwide population coverage

Population Coverage Prediction

The most effective vp7 epitopes depending on affinity rate between HLA molecules have ability to binding and how population coverage include a large area of covering for protection by epitope-HLA binding in individuals the present study revealed the potential vp7 epitopes results according MHC-I interaction of highest population coverage in world region was found in United States 92.09% and Southwest Asia ethnic region was calculated 41.26% which include Iraq table 5, figure 4 this result also refer to implemented epitopes of Rotarix vaccine. Unluckily, no advance particulars about the patient could be reinfected, but there are numerous questions about the possibility of vaccine strain to cove more circulating viral strains would be the cause of disease this opinion is agree with (30) who regarded about different linage of rotavirus strain for example, strains were detected different genetically and antigenically from strains in both Rotarix and RotaTeq vaccines. However, experiments by Computational tools and software, confirmations for these predicted epitopes are indeed necessary for their practical application for created peptides include T-cell and B-cell with specific markers were “ITLYYYQ” vp7 epitopes for B-Cell Epitopes Prediction depending on protein conserved region from Iraq and international legines table 6, table 7 and table 8, figure 5. While another study considered VP7 epitope VMSKRSRSL were most potential

epitopes depending on various physicochemical properties (25). Our study investigated the conserved region with B-cell epitopes because B-cell epitopes have ability to induce both humeral and cellular immunity (24). Together the previous apparatuses T-cell and B-cell epitopes confirmed the presence of antigenicity according epitopes prediction compatible with (22) reveled in an immunological context, it is important to completely aligned of the specific epitope sequence. Bioinformatics tools play an important role in analyzing numerous genomes to choose the defensive epitopes insilico. The cocktails of distinct epitopes or chimeric protein preparations capable to induce atop humeral and cellular immune responses (31). According to epitopes predicted in current study "NPMDITLYYY " with less population coverage 41.26% specially in Southwest Asia demonstrate the inefficiency of Rotarix vaccine, vaccine would be effective for a vast population throughout a wide geographical region only if we certified the antigenicity in two steps, at first the complete proteins were verified for antigenicity and in second round the antigenicity of the potential epitopes were calculated. This provided an exact view of an epitopes antigenicity and its efficacy as a vaccine agent.

5.Conclusion

epitopes recognized in current study "NPMDITLYYY" didn't have ability to cover the whole regions we needed and not at level to give protection against disease in wide regions so this encourage to propose another strategies for epitopes prediction to increase the coverage area accompanied with higher antigenicity and vaccine should contain variant epitopes from variant species such as animal species according the ability of this virus to reassortment.

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