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Original Research Article

Multi Epitope Peptide Vaccine of Capsid Protein against Human Hepatitis E Virus

Mohammed Yousif Mohammed[1], Mona Abdelrahman Mohamed Khaier[2*]

[1]Department of Biotechnology, Faculty of Industrial and Applied Science, University of Bahri, Khartoum, Sudan
[2]Department of Molecular Biology and Bioinformatics, College of Veterinary Medicine, University of Bahri, Khartoum, Sudan

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Abstract: ***Background***: Hepatitis E virus is an inflammation of the liver caused by Hepatitis E virus, which has a faecal- oral transmission route and it infect many people nowadays. ***Aim***: This study aimed to design a vaccine that can cause protection from the Hepatitis E virus mainly in the Capsid protein including the cellular and humeral immunity (B-cell and T-cell epitopes). ***Methods:*** After retrieval of the sequence for the two proteins from National Center for Biotechnology Information (NCBI) BCEpred and ABCpred were used for B cell epitopes. NetMHC 4 serverwas used for T-cell MHC class I. For MHC class II ProPred severs was used. To a identify the antibody prediction epitopes for linear and discontinuous epitopes IEDB Ellipro tool was used. To identify the allergenicity Aller Top server and Vaxijen 2 server were used. For the toxicity Toxinpred was used. A 3D structure modeling was done using I-Tasser server. To visualize the two proteins a Docking process by ClusPro server to predict how HDAg protein binds with the receptor of the immune system. ***Results:*** BCEpred showed 54 epitopes and ABCpred showed 60 epitopes for B cell, for T cell 185 epitopes of NeTMHC 4 MHC class I and 42 epitopes of Propred MHC class II. All epitopes wee antigenic and no allerginecity detected. Also no toxicity detected, allowing to be used as a vaccine showing antibody prediction mode. ***Conclusion:*** In conclusionthis study proposed54 epitopes in the humeral response and 185 in cellular response that could be a powerful multi Capsid epitopes vaccine againsthepatitis E virus. Clinical trial is needed to proof the efficacy of these epitopes as promising candidate vaccine against hepatitis E virus.

Keywords: Hepatitis E virus, NCBI, NetMHC 4, Pro Pred, BCEpred, ABCpred, IEDB, Vaxijen, Ellipro. Allertop, Toxipred, I-tasser, Clus Pro, capsid protein.

INTRODUCTION

Hepatitis E is an inflammation of the liver caused by the infection with the hepatitis E virus (HEV) (Medscape, 2019 and kamar *et al*., 2013). It had a faecal-oral transmission route that is similar to hepatitis A, although the viruses are unrelated (WHO, 2019). In retrospect, the earliest known epidemic of hepatitis E occurred in 1955 in New Delhi (Kumar., *et al*., 2013) but the virus was not isolated until 1983 by Russian scientists investigating an outbreak in Afghanistan (who, 2019). HEV is a positive-sense, single-stranded, non-enveloped, RNA icosahedral virus and one of five known human hepatitis viruses: A, B, C, D, and E. Like hepatitis A, hepatitis E usually follows an acute and self-limiting course of illness (the condition is temporary and the individual recovers) with low death rates in resource-rich areas; however, it can be more severe in pregnant women and people with a weakened immune system, with substantially higher death rates. In pregnant women, especially in the third trimester, the disease is more often severe and is associated with a clinical syndrome called fulminant liver failure, with death rates around 20% (WHO, 2019). Whereas pregnant women may have a rapid and severe course, organ transplant recipients who receive medications to weaken the immune system and prevent organ rejection can develop a slower and more persistent form called chronic hepatitis E, (Zhou X, *et al*. 2013) which is so diagnosed after 3 months of continuous viremia. HEV can be clustered genetically into 8 genotypes, and genotypes 3 and 4 tend to be the ones that cause chronic hepatitis in the immune-suppressedindiviuals. (Kamar, *et al*., 2013). In 2017, hepatitis E was estimated to affect more than 19 million people. (GBD, 2017).Those most commonly at risk of HEV are men aged 15 to 35 years of age. (Zhou X, *et al*., 2013). A preventive vaccine (HEV 239) is approved for use in China. (Li, Shao, *et al*., 2015).

***Corresponding Author:** **Mona A. M. Khaier**

The average incubation period of hepatitis E is 40 days, ranging from 2 to 8 weeks. After a short prodromal phase symptoms may include jaundice, fatigue, and nausea, though most HEV infections are asymptomatic. The symptomatic phase coincides with elevated hepatic aminotransferees levels. (Sanford, *et al*., 2016). Viral RNA becomes detectable in stool and blood serum during the incubation period. Serum IgM and IgG antibodies against HEV appear just before the onset of clinical symptoms. Recovery leads to virus clearance from the blood, while the virus may persist in stool for much longer. Recovery is also marked by disappearance of IgM antibodies and increase of levels of IgG antibodies.

While usually lasting weeks and then resolving, in people with weakened immune systems—particularly in people who have had solid organ transplant—hepatitis E may cause a chronic infection (Bonnet, *et al*., 2012). Occasionally this may result in a life-threatening illness such as fulminant liver failure or liver cirrhosis (Behrendt, *et al*., 2014).

Hepatitis E due to genotypes other than 1 and 2 is thought to be a zoonotic, in that animals are thought to be the primary reservoir; deer and swine have frequently been implicated (Pavio, *et al*., 2010). Domestic animals have been reported as a reservoir for the hepatitis E virus, with some surveys showing infection rates exceeding 95% among domestic pigs (Satou K, *et al*., 2007). Replicative virus has been found in the small intestine, lymph nodes, colon, and liver of experimentally infected pigs. Transmission after consumption of wild deer meat and uncooked deer meat has been reported as well (Li TC, *et al*., 2005). The rate of transmission to humans by this route and the public health importance of this are, however still unclear. A number of other small mammals have been identified as potential reservoirs: the lesser bandicoot rat (Bandicotabengalensis), the black rat (Rattusrattusbrunneusculus) and the Asian house shrew (Suncusmurinus). A new virus designated rat hepatitis E virus has been isolated (Cao, *et al*.2012).

The lifecycle of hepatitis E virus is unknown; the capsid protein obtains viral entry by binding to a cellular receptor. ORF2 (c-terminal) moderates viral entry by binding to HSC70. (Tao, TS, *et al*., 2009). HEV has three open reading frames (ORFs) encoding two polyproteins (ORF1 and ORF2 protein). ORF2 encodes three capsid proteins whereas ORF1 encodes seven fragments involved in viral replication, among others (Balakrishnan, *et al*., 2016). The smallest ORF of the HEV genome, ORF3 is translated from a subgenomic RNA into ORF3, a protein of 113–115 amino acids. ORF3 is proposed to play critical roles in immune evasion by HEV. Previous studies showed that ORF3 is bound to viral particles found in patient sera and produced in cell culture. Although in cultured cells ORF3 has not appeared essential for HEV RNA replication, viral assembly, or infection, it is required for particle release. (Balakrishnan, *et al*., 2016).

MATERIAL AND METHODS

Sequence retrieval

The sequences of the two antigenic protein HDAg and hypothetical were obtained from the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/). The two sequences were applied on different software to select the epitopes that can generate human immune response and can design a vaccine against HepatitisE Virus. The selection and prediction of the epitopes depend on both cellular and humeral immune response that B and T-cells immune cells.

Identification and prediction of B-cell epitopes

In linear B-cell we apply two software servers the ABCpred http://crdd.osdd.net/raghava/abcpred/) (Saha, S and Raghava G.P.S. (2006) and BCpred for conformation result. For ABCpred method it is based on artificial neural network, it relies on random peptides trained on similar B-cell epitopes positive data. Selection of window length 9 result comes out in graphics or tabular frame, adjusting the threshold into +3 to -3, the more threshold lead to better specificity with less sensitivity. The BCpred (http://ailabprojects1.ist.psu.edu:8080/bcpred/index.html) (Saha. S and Raghava G.P.S.2004) use many methods to predict the epitopes (i) our implementation of AAP method (Chen, *et al*., 2007); (ii) BCPred (EL-Manzalawy *et al*., 2008); (iii) FBCPred (EL-Manzalawy *et al*., 2008b). Users provide an antigen sequence and optionally can specify desired epitope length and specificity threshold. Results are returned in several user-friendly formats.

Identification of T-cell epitopes

Using NetMHC 4.0 (http://www.cbs.dtu.dk/services/NetMHC/)(Andreatta and Nielsen,2016) to select T-cell epitopes for HLA-A, B and C in MHC I with length 9 and stronger affinity 0.5 and weak affinity 2 the epitopes come in way that show level binding affinity its also use ANN method for prediction.(Nielsen,*et al*., 2003). Using ProPred (http://crdd.osdd.net/raghava/propred/) (Singh and Raghava, 2001). For T-cell MHC class II binding region in antigen sequence using quantative matrices, the server assist promiscuous binding location regions that are useful selecting vaccine candidates with threshold lower than 3 to show high stringency prediction and default 5% score for binding with MHC class II.

Conservancy Prediction

To assure that these epitopes of B-cells and T-cells are conserve we apply the epitopes of the two cell into conservation tools of IEDB (http:// tools.iedb.org/ conservancy/) and this for linear and discontinuous

sequence with threshold conservancy >100% this help in best selected epitopes.

Antibody Epitope Prediction

Using IEDB Ellipro (http://tools.iedb.org/ellipro/) (Ponomarenko JV, *et al*., 2008) to predict linear and conformational B-cell by using the HDAg protein ElliPro associates each predicted epitope with a score, defined as a PI (Protrusion Index) value averaged over epitope residues. In the method, the protein's 3D shape is approximated by a number of ellipsoids, thus that the ellipsoid with PI = 0.9 would include within 90% of the protein residues with 10% of the protein residues being outside of the ellipsoid; while the ellipsoid with PI = 0.8 would include 80% of residues with 20% being outside the ellipsoid. For each residue, a PI value is defined based on the residue's center of mass lying outside the largest possible ellipsoid; for example, all residues that are outside the 90% ellipsoid will have score of 0.9. Residues with larger scores are associated with greater solvent accessibility. Discontinuous epitopes are defined based on PI values and are clustered based on the distance R (in Å between residue's centers of mass). The larger R is associated with the larger discontinues epitopes being predicted.

Antigenicity, Allerginecity and Toxicity

For conformation of the immunogenic character of all epitopes fragments Vaxijen 2.0 server (http://www.ddg pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) (Doytchinova and Flower, 2007), it's based on the alignment independence method which predicts antigenicity using physiochemical properties and ACC methods for antigenicity assessment peptide fragment with a threshold greater than 0.4 were marked as potentially antigenic.

For allergenicity of selected epitopes and their conscious variants was predicted using Aller Top server (https://www.ddg-pharmfac.net/AllerTOP/). AL Ler TOP uses the auto cross covariance (ACC) method. The server is trained on several known allergies and non-allergens from different species.

For toxicity using Toxin Pred (http://crdd.osdd.net/raghava/toxinpred/) (Sudheer Gupta, *et al*., 2013) is a web server which applies machine learning approaches using different properties of the peptides.

3D structure and Docking

Using I-Tasser server (https://zhanglab.ccmb.med.umich.edu/I-TASSER/), for protein structure and function prediction for the two protein HDAg and hypoheical protein the server predict the structure according to function annotation using protein sequence by multiple approach (Yang *et al*., 2015). For docking ClusPro sever (https://cluspro.bu.edu/login.php)(Dima*et al*.,2017) use to visualize docking for protein using the min protein (HDAg) as target and T-cell receptor as ligand both are protein in nature to see how can they interact. It uses different methods of algorithms.

RESULTS AND DISCUSSION

The BCEpred proposed54 epitopes and ABCpred proposed 60 epitopes for B cell. For T cell 185 epitopes were proposed by NeTMHC 4 software for MHC class I and 42 for MHC class II. The epitopes which are passing MHC class I and class II were 27. All epitopes are antigenic and no allergenicity or toxicity for the selected epitopes.

Identification of B cell epitopes

Using BCEpred result showed 54 epitopes, and 60 epitopes using ABCpred all epitopes are 100% conserves using IEDB conservation tools and cangenerate immune response table (1) show result of BCEpred and Table (2) show result of ABC pred.

Table-1: Result of BCEpred

Rank	epitopes	Conservancy
1	PGQPSGRRRGRRSGGSGGG	C
2	GDRADSQP	C
3	RDQAQRPA	C
4	SRRRPTT	C
5	PDVDSRGA	C
6	QDGTNTH	C
7	QTTTTPTS	C
8	NSITSTD	C
9	RSVETSGVAEEEATSG	C
10	TPGNTNTRVS	C
11	RRGADGTAE	C
12	TSTNGVGE	C
13	SANGEPT	C
14	SVENAQQDKG	C
15	QDYDNQHEQDRPTPSP	C

16	TAAEYDQSTYGSSTG	C
17	STTQQYSKT	C
18	EAGTTKAG	C
19	YNTTASDQ	C
20	PAPPPGQPSGRRRGRRSGG	C
21	GFWGDRA	C
22	RPAAASRRRP	C
23	PVPDVDS	C
24	QGWRSVET	C
25	LTPGNTNTRVSRYS	C
26	HRLRRGA	C
27	ELISSAG	C
28	HEQDRPT	C
29	YDQSTYGSST	C
30	LKMKVGK	C
31	PAPPPGQPSGRRRGRRSGG	C
32	GDRADSQPF	C
33	AGPRVRQPARPL	C
34	AWRDQAQRPAAASRRRPTTAG	C
35	PAHDTPPVPD	C
36	ILRRQYNLSTS	C
37	QDGTNTH	C
38	EASNYAQYRV	C
39	RATIRYRPLVPN	C
40	WPQTTTTPTS	C
41	PSERLHYRNQGWRS	C
42	AEEEATSG	C
43	VNSYTNTPYTG	C
44	EFRNLTPGNTNTRVSRYSSTARHRLRRGADGT	C
45	ATRFMKD	C
46	NGEPTVKLY	C
47	SVENAQQDKG	C
48	IQDYDNQHEQDRPTPSPAPSRP	C
48	TAAEYDQSTYGS	C
50	RSLDWTK	C
51	RPLSTTQQYSKTF	C
52	TTKAGYPYNYNTTASDQ	C
53	EDTMDYPARAHTFDD	C
54	AELQRLKMKVGKTRE	C

Table-2: Result of BCEpred epitopes

Rank	Sequence	Start position	Score	conservency
1	ARPLGSAWRD	80	0.83	C
2	VETSGVAEEE	261	0.82	C
3	GDRADSQPFA	43	0.81	C
4	ATRFMKDLYF	346	0.79	C
4	QDGTNTHIMA	167	0.79	C
5	HGSLVNSYTN	281	0.78	C
6	ERLHYRNQGW	249	0.77	C
6	VLYAAPLSPL	154	0.77	C
7	ARHRLRRGAD	327	0.76	C
7	LVMLCIHGSL	275	0.76	C
8	YRPLVPNAVG	194	0.75	C
8	MFLPMLPAPP	12	0.75	C
8	VAPAHDTPPV	113	0.75	C
9	IPYIHPTNPF	53	0.74	C

9	AGAAPLTAVA	105	0.74	C
10	HPTNPFAPDV	57	0.73	C
10	SITSTDVRIL	226	0.73	C
10	LPAPPPGQPS	17	0.73	C
11	AGAGPRVRQP	70	0.72	C
11	STNGVGEIGR	357	0.72	C
11	PVPDVDSRGA	121	0.72	C
12	PFAPDVTAAA	61	0.7	C
12	ASNYAQYRVV	179	0.7	C
13	GGFWGDRADS	39	0.69	C
13	ILRRQYNLST	131	0.69	C
14	SQPFAIPYIH	48	0.68	C
14	LFNLADTLLG	372	0.68	C
14	GEIGRGIALT	362	0.68	C
14	TNTRVSRYSS	316	0.68	C
14	RRRGRRSGGS	28	0.68	C
14	EEEATSGLVM	268	0.68	C
14	TTTTPTSVDM	215	0.68	C
15	EPTVKLYTSV	407	0.65	C
15	QLFYSRPVVS	394	0.65	C
15	GGYAISISFW	203	0.65	C
15	APLSPLLPLQ	158	0.65	C
16	FRNLTPGNTN	308	0.64	C
17	RRRPTTAGAA	99	0.63	C
18	ISSAGGQLFY	388	0.62	C
18	GTAELTTTAA	337	0.62	C
18	RNQGWRSVET	254	0.62	C
18	HVIPSERLHY	244	0.62	C
18	MRPRPILLLL	1	0.62	C
19	PVVSANGEPT	400	0.6	C
19	TLLGGLPTEL	378	0.6	C
19	MKDLYFTSTN	350	0.6	C
19	SGGSGGGFWG	34	0.6	C
19	QPGIASEHVI	237	0.6	C
19	ISISFWPQTT	207	0.6	C
19	SVATGTNLVL	146	0.6	C
20	LGLLDFALEL	297	0.59	C
20	SYTNTPYTGA	287	0.59	C
20	TDVRILVQPG	230	0.59	C
21	AWRDQAQRPA	86	0.58	C
21	VPNAVGGYAI	198	0.58	C
22	NLSTSPLTSS	137	0.57	C
23	GLPTELISSA	382	0.56	C
24	RGADGTAELT	333	0.55	C
25	AQYRVVRATI	183	0.54	C
26	ALELEFRNLT	303	0.53	C
26	PPGQPSGRRR	21	0.53	C
27	THIMATEASN	172	0.52	C

Identification of T cell epitopes

Using NeTMHC 4 server for MHC class I result showed that 185epitopes and Pro Pred for MHC class II result showed 42epitopes, all are 100% conserves using IEDB tools .After selection of epitopes and removal ofduplicates that can generate immune response. Table (3) and (4) show the two software results

Table-3: Results of Epitopes predicted by NeTMHC4 server for MHC class I

alleles	epitopes	affinity	rank	allele	epitopes	affinity	rank	allele	epitopes	affinity	rank	allel	epitopes	affinity	rank
HLA-A0101	SSAGGQLFY	0.731	0.03		LYAAPLSPL	0.791	0.1		TPYTGALGL	0.597	0.4		MATEASNYA	0.553	0.5
	NTNTRVSRY	0.648	0.08		RFMKDLYFT	0.574	0.5		LPLRGKLSF	0.727	0.09	HLA-B3503	HPTNPFAPD	0.149	0.5
	ATGTNLVLY	0.612	0.12		GYPYNYNTT	0.714	0.2		YPYNYNTTA	0.599	0.4		SPLTSSVAT	0.162	0.5
	WLSLTAAEY	0.526	0.2		AFQSTVAEL	0.693	0.25		APHSALALL	0.82	0.02		APSRPFSVL	0.17	0.4
	DSQPFAIPY	0.475	0.3	HLA-A2501	STSPLTSSV	0.238	0.4		.			HLA-B3901	WRSVETSGV	0.603	0.15
	TTASDQLLV	0.475	0.3		LVPNAVGGY	0.258	0.4	HLA-B0802	GPRVRQPAR	0.144	0.4	HLA-B4001	AEEEATSGL	0.776	0.05
HLA-A0201	GLLDFALEL	0.393	0.5		PTSVDMNSI	0.308	0.2		AILRRQYNL	0.15	0.3		GEIGRGIAL	0.88	0.01
	YVSDSVTLV	0.808	0.08		ESRVVIQDY	0.246	0.4		IPSERLHYR	0.137	0.4	HLA-B4002	LEFRNLTPG	0.57	0.4
	QQYSKTFFV	0.766	0.15		TTQQYSKTF	0.227	0.5		YSSTARHRL	0.158	0.25	HLA-C0303	IAIPHDIDL	0.81	0.06
	ILLLLLMFL	0.696	0.4	HLA-A2602	TTTAATRFM	0.317	0.7		CPECRPLGL	0.187	0.12		VATGTNLVL	0.711	0.17
HLA-A0202	LLGGLPTEL	0.798	0.2		STVAELQRL	0.27	1	HLA-B0803	AQYRVVRAT	0.294	0.02		RGADGTAEL	0.684	0.2
	VLAPHSALA	0.768	0.3	HLA-A2603	PTTAGAAPL	0.266	0.5	HLA-B1402	MRPRPILLL	0.303	0.5	HLA-C0401	SFWEAGTTK	0.332	0.01
	AISTYTTSL	0.735	0.5		DVDSRGAIL	0.295	0.4		DQAQRPAAA	0.335	0.3		LFNLADTLL	0.3	0.04
HLA-A0203	KLYTSVENA	0.786	0.3		YTNTPYTGA	0.287	0.4		AAASRRRPT	0.324	0.4		TFDDFCPEC	0.293	0.04
	MLCIHGSLV	0.786	0.3		ELISSAGGQ	0.411	0.09		AASRRRPTT	0.324	0.4		FWEAGTTKA	0.265	0.08
	SVATGTNLV	0.772	0.4		VVIQDYDNQ	0.3	0.4		SRRRPTTAG	0.38	0.15		SFWPQTTTT	0.243	0.15
HLA-A0205	AVGGYAISI	0.381	0.17		WTKVTLDGR	0.357	0.17		AGAAPLTAV	0.3	0.5	HLA-C0501	RADSQPFAI	0.702	0.05
	NLVLYAAPL	0.332	0.3		GTTKAGYPY	0.337	0.25		TRVSRYSST	0.304	0.5		LADTLLGGL	0.664	0.07
	SLGAGPVSI	0.302	0.4		AVLAPHSAL	0.267	0.5		TAELTTTAA	0.304	0.5		LQDGTNTHI	0.533	0.17
	PTVKLYTSV	0.301	0.4		HTFDDFCPE	0.304	0.4		TRFMKDLYF	0.294	0.5		VSDSVTLVN	0.42	0.4
HLA-A0206	FAIPYIHPT	0.801	0.15	HLA-A2902	IMATEASNY	0.717	0.17		LAPHSALAL	0.315	0.4	HLA-C0602	LRRQYNLST	0.38	0.4
	SQPFAIPYI	0.764	0.25		YGSSTGPVY	0.624	0.4	HLA-B1502	VNSYTNTPY	0.358	0.5	HLA-C0701	SRYSSTARH	0.338	0.5
	FAPDVTAAA	0.728	0.4		TKAGYPYNY	0.625	0.4	.	ISSAGGQLF	0.36	0.5	HLA-C0702	FYSRPVVSA	0.332	0.5
	GQLFYSRPV	0.722	0.4		ALLEDTMDY	0.632	0.4		PSPAPSRPF	0.413	0.3	HLA-C0802	FSVLRANDV	0.325	0.15
	YAAPLSPLL	0.702	0.5	HLA-A3001	YIHPTNPFA	0.713	0.17		TQQYSKTFF	0.403	0.4	HLA-C1203	NAAGHRVAI	0.637	0.17
HLA-A0207	QLFYSRPVV	0.163	0.3		RVRQPARPL	0.842	0.03		PLGLQGCAF	0.396	0.4		YSRPVVSAN	0.562	0.4
HLA-A0211	LMFLPMLPA	0.862	0.17		ASRRRPTTA	0.726	0.15		RPLSTTQQY	0.459	0.2		YAQYRVVRA	0.507	0.5

.	VMLCIHGSL	0.907	0.06		RRRPTTAGA	0.631	0.4	HLA-B1509	THIMATEAS	0.365	0.15	HLA-C1402	TYGSSTGPV	0.664	0.12
	FMKDLYFTS	0.84	0.25		VVRATIRYR	0.611	0.4		EHVIPSERL	0.495	0.04		LYFTSTNGV	0.637	0.17
	SLDWTKVTL	0.836	0.25		ATIRYRPLV	0.617	0.15		LHYRNQGWR	0.275	0.4	HLA-C1502	ASNYAQYRV	0.532	0.15
HLA-A0212	AQQDKGIAI	0.637	0.5		RYRPLVPNA	0.872	0.02		TNTPYTGAL	0.264	0.4		PTNPFAPDV	0.53	0.15
HLA-A0216	LLLMFLPM	0.626	0.5		VSRYSSTAR				YNTTASDQL	0.246	0.5		RSVETSGVA	0.507	0.2
	TLFNLADTL	0.718	0.25		RGKLSFWEA	0.617	0.4		MKVGKTREL	0.315	0.25		TSTDVRILV	0.501	0.2
HLA-A0217	LLMFLPMLP	0.766	0.01	HLA-A3002	RVVRATIRY	0.578	0.4	HLA-B1517	RSGGSGGGF	0.827	0.17		TTTPTSVDM	0.486	0.25
	MFLPMLPAP	0.55	0.25		IHGSLVNSY	0.544	0.5		SSVATGTNL	0.747	0.4		QTTTTPTSV	0.462	0.3
	MLPAPPPGQ	0.482	0.4		ATRFMKDLY	0.613	0.25		LTTTAATRF	0.869	0.1		RSLDWTKVT	0.419	0.5
	ALGLLDFAL	0.47	0.5	HLA-A3101	RYSSTARHR	0.742	0.17		QAVARSLDW	0.742	0.4				
HLA-A0250	KLSFWEAGT	0.704	0.4		SSTARHRLR	0.769	0.12		VSISAVAVL	0.752	0.4				
	GLQGCAFQS	0.678	0.5		TARHRLRRG	0.56	1	HLA-B1801	TEASNYAQY	0.685	0.12				
HLA-A0301	TTAATRFMK	0.631	0.25		PSRPFSVLR	0.641	0.4		EEATSGLVM	0.564	0.3				
	KTFFVLPLR	0.611	0.3	HLA-A3201	RQYNLSTSP	0.613	0.15	HLA-B2705	YRVVRATIR	0.586	0.4				
HLA-A1101	STARHRLRR	0.606	0.5		RLHYRNQGW	0.642	0.1		VRILVQPGI	0.586	0.4				
HLA-A2301	PYIHPTNPF	0.689	0.12	HLA-B0702	RPRPILLLL	0.809	0.03		LRANDVLWL	0.602	0.4				
	QYRVVRATI	0.669	0.15		RPILLLLLM	0.809	0.03		QRLKMKVGK	0.567	0.5				
	GYAISISFW	0.554	0.4		QPARPLGSA	0.617	0.3	HLA-B2720	RRGRRSGGS	0.481	0.5				
	VYVSDSVTL	0.6	0.3		APLTAVAPA	0.586	0.4		RRPTTAGAA	0.515	0.4				
	QYSKTFFVL	0.524	0.5		APAHDTPPV	0.82	0.02		YRPLVPNAV	0.679	0.12				
HLA-A2402	DYPARAHTF	0.646	0.08		APLSPLLPL	0.82	0.02		YRNQGWRSV	0.857	0.01				
HLA-A2403	QYNLSTSPL	0.868	0.04		RATIRYRPL	0.55	0.5		SKTFFVLPL	0.491	0.5				
	WPQTTTTPT	0.664	0.2	HLA-B3501	QPFAIPYIH	0.625	0.3								

Table-4: Results of Epitopes predicted by ProPred for MHC class II

Allele	Epitopes	conservancy
DRB1_0101	LVLYAAPLS	C
	YRPLVPNAV	C
	YRVVRATIR	C
	MNSITSTD	C
	YRNQG	C
	WRSVETSGV	C
	MLCIHGSLV	C
	FRNLTPGNT	C
	IGRGIALTL	C
	VVSANGEPT	C
	LRANDVL	C
	VLPLRGKLS	C
	VAVLAPHSA	C

DRB1_0102	PMLPAPPP	C
	LRRQYNLST	C
	VLYAAPLSP	C
	ILVQPGIAS	C
	LLGGLPTEL	C
	LFYSRP	C
	VNVATGAQA	C
	VENAAGHRV	C
DRB1_0301	ILLLLLMFL	C
	VRQPARPLG	C
	VVRATIRYR	C
	LVPNAVGGY	C
	VRILVQPGI	C
	VIPSERLHY	C
	LVNSYTNTP	C
	YVSDSVT	C
	LVNVATGAQ	C
	VTLDGRPLS	C
	VSISAVAVL	C
	MLPAPPPGQ	C
	FWGDRADSQ	C
	FAPDVTAAA	C
	YNLSTSPLT	C
	LVMLCIHGS	C
	FMKDLYFTS	C
	FNLADTLLG	C
	FVLPLRGKL	C
	LGAGPVSIS	C
DRB1_0306	VVIQDYDNQ	C

Antibody Epitope Prediction

Using ELLipro software from IEDB with PDB ID(3RKD) showed that 26 predicted linear epitopes table (5) show the result and 8 discontinuous epitopes table (6) show the result

Table-5: Result of linear epitopes

NO	CHAIN	START	END	PEPTIDE	NUMBER OF RESIDUES	SCORE
1	A	514	571	LDWTKVTLDGRPLSTIQQHSKTFFVLPLRGKLSFWEAGTTKAG YPYNYNTTASDQLLV	58	0.797
2	A	578	604	RVAISTYTTSLGAGPVSISAVAVLAPP	27	0.776
3	A	459	494	SRPFSVLRANDVLWLSLTAAEYDQSTYGSSTGPVYV	36	0.741
4	A	497	509	SVTLVNVATGAQA	13	0.723
5	B	514	571	LDWTKVTLDGRPLSTIQQHSKTFFVLPLRGKLSFWEAGTTKAG YPYNYNTTASDQLLV	58	0.79
6	B	578	604	RVAISTYTTSLGAGPVSISAVAVLAPP	27	0.762
7	B	497	509	SVTLVNVATGAQA	13	0.731
8	B	460	494	RPFSVLRANDVLWLSLTAAEYDQSTYGSSTGPVYV	35	0.722
9	L	177	213	STLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNE	37	0.88
10	L	139	161	FYPKDINVKWKIDGSERQNGVLN	23	0.757
11	L	107	136	KRADAAPTVSIFPPSSEQLTSGGASVVCFL	30	0.711
12	L	24	29	RASEII	6	0.526
13	H	219	224	KKIVPR	6	0.659
14	H	194	204	VPSSTWPSETV	11	0.622
15	H	13	17	QPSQT	5	0.613
16	H	123	149	SSAKTTPPSVYPLAPGSMVTL	21	0.597
17	H	182	185	QSDL	4	0.587
18	H	65	68	LKSR	4	0.528
19	C	175	213	MSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNE	39	0.872
20	C	107	161	KRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKI DGSERQNGVLN	55	0.744
21	C	12	31	SVSVGETVTITCRASEIIYS	20	0.537
22	C	74	78	KINSL	5	0.505
23	D	219	224	KKIVPR	6	0.672
24	D	193	204	TVPSSTWPSETV	12	0.662
25	D	130	149	PSVYPLAPGSMVTL	14	0.633
26	D	13	16	QPSQ	4	0.538

Table-6: Results of discontinuous epitopes

No.	Residues	Number of residues	Score
1	A:S459, A:R460, A:P461, A:F462, A:S463, A:V464, A:L465, A:R466, A:A467, A:N468, A:D469, A:V470, A:L471, A:W472, A:L473, A:S474, A:L475, A:T476, A:A478, A:E479, A:Y480, A:D481, A:Q482, A:S483, A:T484, A:Y485, A:G486, A:S487, A:S488, A:T489, A:G490, A:P491, A:V492, A:Y493, A:V494, A:S497, A:V498, A:T499, A:L500, A:V501, A:N502, A:V503, A:A504, A:T505, A:G506, A:A507, A:Q508, A:A509, A:A511, A:L514, A:D515, A:W516, A:T517, A:K518, A:V519, A:T520, A:L521, A:D522, A:G523, A:R524, A:P525, A:L526, A:S527, A:T528, A:I529, A:Q530, A:Q531, A:H532, A:S533, A:K534, A:T535, A:F536, A:F537, A:V538, A:L539, A:P540, A:L541, A:R542, A:G543, A:K544, A:L545, A:S546, A:F547, A:W548, A:E549, A:A550, A:G551, A:T552, A:T553, A:K554, A:A555, A:G556, A:Y557, A:P558, A:Y559, A:N560, A:Y561, A:N562, A:T563, A:T564, A:A565, A:S566, A:D567, A:Q568, A:L569, A:L570, A:V571, A:N573, A:H577, A:V579, A:A580, A:I581, A:S582, A:T583, A:Y584, A:T585, A:T586, A:S587, A:L588, A:G589, A:A590, A:G591, A:P592, A:V593, A:S594, A:I595, A:S596, A:A597, A:V598, A:A599, A:V600, A:L601, A:A602, A:P603, A:P604, H:D56, H:T105, L:W92, L:G93, L:N94	140	0.754
2	C:R108, C:A109, C:D110, C:A111, C:A112, C:P113, C:T114, C:V115, C:S116, C:I117, C:F118, C:P119, C:P120, C:S121, C:S122, C:E123, C:Q124, C:L125, C:T126, C:S127, C:G128, C:G129, C:A130, C:S131, C:V132, C:V133, C:C134, C:F135, C:L136, C:N137, C:N138, C:F139, C:Y140, C:P141, C:K142, C:D143, C:I144, C:N145, C:V146, C:K147, C:W148, C:K149, C:I150, C:D151, C:G152, C:S153, C:E154, C:R155, C:Q156, C:N157, C:G158, C:V159, C:L160, C:N161, C:S171, C:T172, C:M175, C:S177, C:T178, C:L179, C:T180, C:L181, C:T182, C:K183, C:D184, C:E185, C:Y186, C:E187, C:R188, C:H189, C:N190, C:S191, C:Y192, C:T193, C:C194, C:E195, C:A196, C:T197, C:H198, C:K199, C:T200, C:S201, C:T202, C:S203, C:P204, C:I205, C:V206, C:K207, C:S208, C:F209, C:N210, C:R211, C:N212, C:E213, D:P130, D:S131, D:Y133, D:P134, D:L135, D:A136, D:P137, D:G138, D:S145, D:M146, D:V147, D:T148, D:L149, D:K154, D:N166, D:G173, D:T193, D:V194, D:P195, D:S196, D:S197, D:T198, D:W199, D:P200, D:S201, D:E202, D:T203, D:V204, D:K219, D:K220, D:I221, D:V222, D:P223, D:R224	128	0.752
3	B:R460, B:P461, B:F462, B:S463, B:V464, B:L465, B:R466, B:A467, B:N468, B:D469, B:V470, B:L471, B:W472, B:L473, B:S474, B:L475, B:T476, B:A478, B:E479, B:Y480, B:D481, B:Q482, B:S483, B:T484, B:Y485, B:G486, B:S487, B:S488, B:T489, B:G490, B:P491, B:V492, B:Y493, B:V494, B:S497, B:V498, B:T499, B:L500, B:V501, B:N502, B:V503, B:A504, B:T505, B:G506, B:A507, B:Q508, B:A509, B:A511, B:L514, B:D515, B:W516, B:T517, B:K518, B:V519, B:T520, B:L521, B:D522, B:G523, B:R524, B:P525, B:L526, B:S527, B:T528, B:I529, B:Q530, B:Q531, B:H532, B:S533, B:T535, B:F536, B:F537, B:V538, B:L539, B:P540, B:L541, B:R542, B:G543, B:K544, B:L545, B:S546, B:F547, B:W548, B:E549, B:A550, B:G551, B:T552, B:T553, B:K554, B:A555, B:G556, B:Y557, B:P558, B:Y559, B:N560, B:Y561, B:N562, B:T563, B:T564, B:A565, B:S566, B:D567, B:Q568, B:L569, B:L570, B:V571, B:N573, B:H577, B:V579, B:A580, B:I581, B:S582, B:T583, B:Y584, B:T585, B:T586, B:S587, B:L588, B:G589, B:A590, B:G591, B:P592, B:V593, B:S594, B:I595, B:S596, B:A597, B:V598, B:A599, B:V600, B:L601, B:A602, B:P603, B:P604, C:I2, C:R24, C:A25, C:S26, C:E27, C:I28, C:I29, C:Y30, C:A51, C:S65, C:G66, C:S67, C:G68, C:T69, C:Q70, C:W92, C:G93, C:N94, D:T105	152	0.728
4	H:Q13, H:P14, H:S15, H:Q16, H:T17, H:L65, H:K66, H:S67, H:R68, H:A85, H:S86, H:V87, H:S123, H:S124, H:A125, H:K126, H:T127, H:T128, H:P129, H:P130, H:S131, H:Y133, H:P134, H:L135, H:A136, H:P137, H:G138, H:S145, H:M146, H:V147, H:T148, H:L149, H:K154, H:G155, H:F157, H:Q182, H:S183, H:D184, H:L185, H:V194, H:P195, H:S196, H:S197, H:T198, H:W199, H:P200, H:S201, H:E202, H:T203, H:V204, H:K219, H:K220, H:I221, H:V222, H:P223, H:R224, L:R108, L:A109, L:D110, L:A111, L:A112, L:P113, L:T114, L:V115, L:S116, L:I117, L:F118, L:P119, L:P120, L:S121, L:S122, L:E123, L:Q124, L:L125, L:T126, L:S127, L:G128, L:G129, L:A130, L:S131, L:V132, L:V133, L:C134, L:F135, L:L136, L:F139, L:Y140, L:P141, L:K142, L:D143, L:I144, L:N145, L:V146, L:K147, L:W148, L:K149, L:I150, L:D151, L:G152, L:S153, L:E154, L:R155, L:Q156, L:N157, L:G158, L:V159, L:L160, L:N161, L:S171, L:T172, L:S177, L:T178, L:L179, L:T180, L:L181, L:T182, L:K183, L:D184, L:E185, L:Y186, L:E187, L:R188, L:H189, L:N190, L:S191, L:Y192, L:T193, L:C194, L:E195, L:A196, L:T197, L:H198, L:K199, L:T200, L:S201, L:T202, L:S203, L:P204, L:I205, L:V206, L:K207, L:S208, L:F209, L:N210, L:R211, L:N212, L:E213	147	0.714
5	C:S12, C:V13, C:S14, C:V15, C:G16, C:E17, C:T18, C:V19, C:T20, C:R61, C:K74, C:N76, C:S77, C:L78, C:K107	15	0.561
6	D:Q182, D:S183, D:D184	3	0.555
7	L:I2, L:R24, L:S26, L:E27, L:I28, L:I29, L:Y30, L:S67, L:G68, L:T69, L:Q70	11	0.522
8	C:S7, C:P8, C:T22	3	0.502

Antigenicity, allergenicity and toxicity of Epitopes

Using vaixjen server result showed capsid protein of hepatitis E virus is antigenic with default protective antigen 0.6018 and no allergy using ALlerTope, and no toxicity present using Toxin Pred server.

3D structure and Docking

The capsid protein of hepatitis E virus was showed as 3D structure using I-Tasser server figure (1) showed the result and docking using Clus Pro server with the MHC I receptors result of docking showed on figure (2).

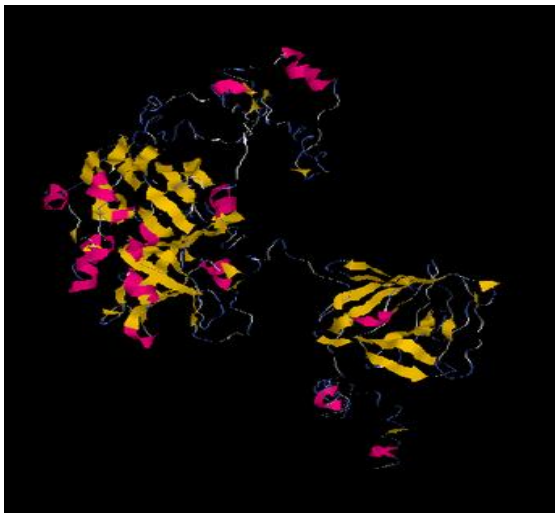

Fig-1: show 3D structure of capsid protein

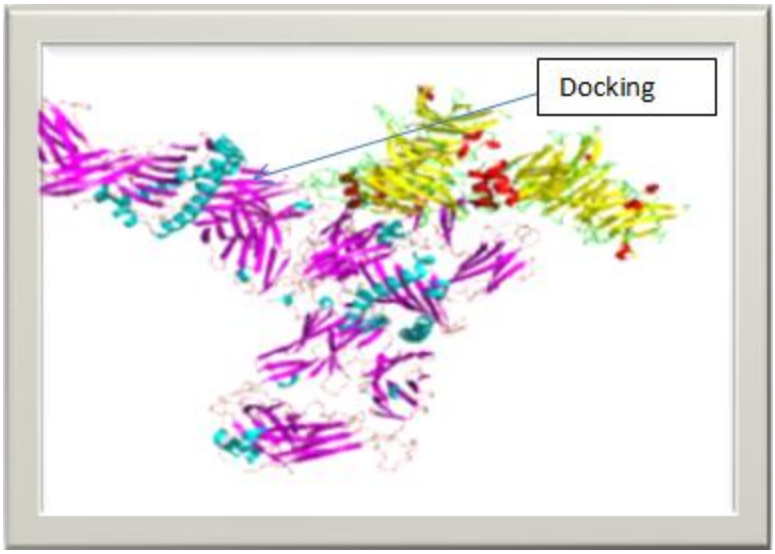

Fig-2: result of docking between capsid protein and its binding MHC receptor

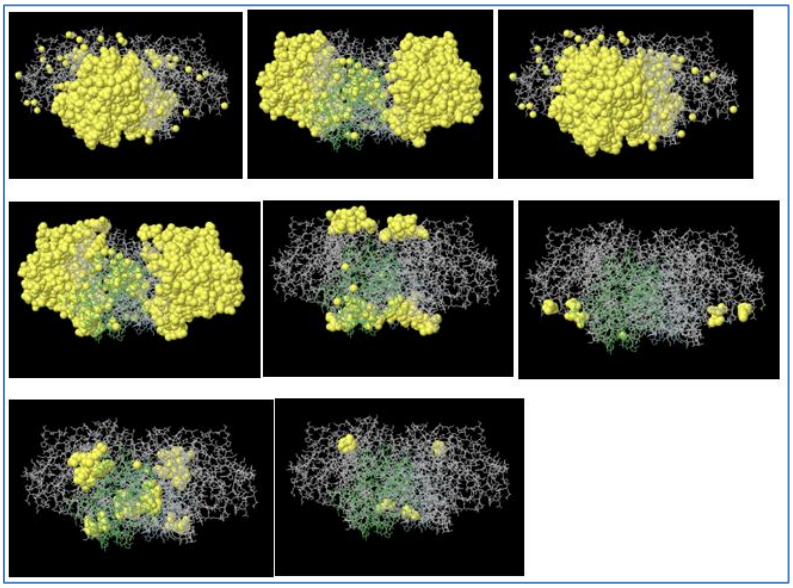

Fig-3: Result of discontinuous epitope structure

DISCUSSION

In recent years, there have been numerous outbreaks caused by HEV genotype 1 in camps for displaced persons (refugees) in Africa, resulting in substantial morbidity and mortality. Persons living in such camps may not have adequate access to clean water and sanitation, leading to high risk of exposure to an infectious dose of the virus [30]. This study designed and predicted an epitope for Hepatitis E Virus targeting the capsid protein. Hepatitis E Virus causes a liver disease, the virus is not that virulent but it causes serious damage to the liver considering its transmission and contiguity. No vaccine was designed using bioinformatics tools. Most studies focus on hepatitis B virus due to its serious problems to the liver ignoring E virus. In fact E virus infect young people and it can be treated using some medicines but still vaccination is the best cure way to prevent spreading of the infection among children [31]. The most importance its invasive to pregnant women that let us to focus more on finding protection before cure most studies in hepatitis E virus focus on wet-lab procedure to get best vaccine or medicine and if they do they generalized the whole genome virus not like this study focus on the external features of the virus which is the capsid protein that once the virus enter the blood stream it can generate immune response.

RECOMMENDATION

After showing the epitopes presented in this study we recommend to design this epitopes and to conduct clinical trials to get the best vaccine, besides doing more application on different areas and ethnicity groups due to its different genetic maps.

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