WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 10, 1023-1040. Research Article ISSN 2277– 7105

VACCINE DESIGN FOR HD AG-L AND HYPOTHETICAL PROTEIN OF HEPATITIS DELTA VIRUS

Mohammed Yousif Mohammed[1] and Mona 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.

Article Received on
21 June 2021,

Revised on 12 July 2021,
Accepted on 02 August 2021

DOI: 10.20959/wjpr202110-20577

***Corresponding Author**
Mona Mohamed Khaier
Department of Molecular Biology and Bioinformatics, College of Veterinary Medicine, University of Bahri, Khartoum, Sudan.

ABSTRACT

Background: Hepatitis Delta Virus is responsible for many complications occurring during infection of Hepatitis B virus and responsible for serious damage to liver, most studies focus only on hepatitis B virus ignoring delta virus but its co-infection to liver make it very important in studies and research. ***Aim:*** in this study an epitopes vaccine was designed for Hepatitis Delta Virus (HDV) of the two protein present HD Ag and hypothetical protein using bioinformatics prediction tools. ***Methods*:** After retrieval of the two protein sequences from National Center for Biotechnology Information (NCBI) BCEpred and ABCpred for B cell epitopes and NetMHC 4 server for T-cell MHC class I and MHC class II was used. ProPred sever was used also to identify the antibody prediction epitopes for linear and discontinuous epitopes using IEDB Ellipro tool. Allergenicity was predicted by AllerTop server, antigenicity by Vaxijen 2 server and toxicity was predicted using Toxinpred. The 3D structure was modeled using I-Tasser server to visualize the two proteins. Docking process was achieved by ClusPro server to see how HDAg protein binds with the receptor of immune system. ***Results:*** BCEpred predicted 5 epitopes and ABCpred predicted 21 epitopes for B cell of HD Ag protein for hypothetical predicted 10 BCEpred epitopes and 11 ABCpred epitopes for hypothetical protein for T cell 16 epitopes were predicted by NeTMHC 4 MHC class I and 12 epitopes for MHC class II for ProPred of HDAg protein and 15 epitopes for MHC class I and 9 epitopes for MHC class II for hypothetical protein. All epitopes are antigenic

and no allergy was detected plus not toxic effect was predicted. Concluding that these predicted epitopes can be used as a multi epitopes vaccine.

INTRODUCTION

Hepatitis D is a type of viral hepatitis (*"Hepatitis (Viral) NIDDK".2020)* caused by the hepatitis delta virus (HDV), a small spherical enveloped particle that shares similarities with both a viroidandvirusoid(*Farci P (2003))(MagniusL,et al,2018)*HDV is one of five known hepatitis viruses: A, B, C, D, and E. HDV is considered to be a satellite. Because it can propagate only in the presence of the hepatitis B virus (HBV).(Makino *et al.*, 1987*)* Transmission of HDV can occur either via simultaneous infection with HBV (co infection) or superimposed on chronic hepatitis B or hepatitis B carrier state (super infection).

HDV and HBV infecting a person simultaneously is considered the most serious type of viral hepatitis due to its severity of complications. (*"Hepatitis D". www.who.int. Retrieved 2020-09-20)*These complications include a greater likelihood of experiencing liver failure in acute infections and a rapid progression to liver cirrhosis, with an increased risk of developing liver cancer in chronic infections.(*FattovichG,et al, 2000)*In combination with hepatitis B virus, hepatitis D has the highest fatality rate of all the hepatitis infections, at 20%. The HDV (hepatitis delta virus) is a small, spherical virus with a 36 nm diameter. It has anviral envelope containing host phospholipids and three kinds of HBV envelope protein – large, medium, and small hepatitis B surface antigens; this surrounds an inner nucleocapsid. The nucleocapsid contains the genome surrounded by about 200 molecules of hepatitis D antigen (HDAg) for each genome. The central region of HDAg has been shown to bind RNA.(*Poisson F,et al, 1993)*Several interactions are also mediated by a coiled-coil region at the N terminus of HDAg.(*ZuccolaHJ, et al, 1998).*

The HDV genome is negative sense, single-stranded, closed circular RNA; with a genome of approximately 1700 nucleotides, HDV is the smallest "virus" known to infect animals. It has been proposed that HDV may have originated from a class of plant pathogens called viroids, which are much smaller than viruses. (*Elena SF,et al,* 1991)(Sureau, 2006). Its genome is unique among animal viruses because of its high GC nucleotide content. Its nucleotide sequence is about 70% self-complementary, allowing the genome to form a partially double-stranded, rod-like RNA structure. (*SaldanhaJA,et al, 1990).*

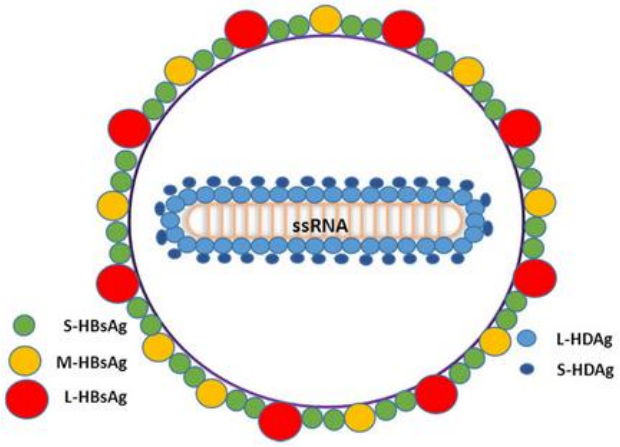


Figure 1: Structure of Hepatitis D virus (*Sureau, 2006*).

Like hepatitis B, HDV gains entry into liver cells via the NTCP(*Yan H,et al, 2012)*bile transporter. HDV recognizes its receptor via the N-terminal domain of the large hepatitis B surface antigen, HBsAg.(*EngelkeM,et al, 2016)*Mapping by mutagenesis of this domain has shown that amino acid residues 9–15 make up the receptor-binding site.[18] After entering the hepatocyte, the virus is uncoated and the nucleocapsidtranslocated to the nucleus due to a signal in HDAg(*Xia YP,et al, 1992)* Since the nucleocapsid does not contain an RNA polymerase to replicate the virus' genome, the virus makes use of the cellular RNA polymerases. (*Lehmann E,et al, 2007)* Initially thought to use just RNA polymerase II, now RNA polymerases I and III have also been shown to be involved in HDV replication.(*Greco-Stewart VS,et al, 2009)* Normally RNA polymerase II utilizes DNA as a template and produces mRNA. Consequently, if HDV indeed utilizes RNA polymerase II during replication, it would be the only known animal pathogen capable of using a DNA-dependent polymerase as an RNA-dependent polymerase, (Greco-Stewart, *et al*,, 2009*).*

The RNA polymerases treat the RNA genome as double-stranded DNA due to the folded rod-like structure it is in. Three forms of RNA are made; circular genomic RNA, circular complementary antigenomic RNA, and a linear polyadenylatedantigenomic RNA, which is the mRNA containing the open reading frame for the HDAg. Synthesis of antigenomic RNA occurs in the nucleolus, mediated by RNA polymerase I, whereas synthesis of genomic RNA takes place in the nucleoplasm, mediated by RNA polymerase II.(*Li YJ, MacnaughtonTet al, 2006)*HDV RNA is synthesized first as linear RNA that contains many copies of the genome. The genomic and antigenomic RNA contain a sequence of 85 nucleotides, the hepatitis delta virus ribozyme, that acts as a ribozyme, which self-cleaves the linear RNA into monomers. These monomers are then ligated to form circular RNA.(*Branch AD,,et al, 1989).*

A significant difference between viroids and HDV is that, while viroids produce no proteins, HDV is known to produce one protein, namely HDAg. It comes in two forms; a 27kDa large-HDAg, and a small-HDAg of 24kDa. The N-terminals of the two forms are identical; they differ by 19 more amino acids in the C-terminal of the large HDAg.(*Weiner AJ,et al 1988)*Both isoforms are produced from the same reading frame which contains an UAG stop codon at codon 196, which normally produces only the small-HDAg. However, editing by cellular enzyme adenosine deaminase-1 changes the stop codon to UGG, allowing the large-HDAg to be produced. (Weiner *et al.*, 1988) Despite having 90% identical sequences, these two proteins play diverging roles during the course of an infection. HDAg-S is produced in the early stages of an infection and enters the nucleus and supports viral replication. HDAg-L, in contrast, is produced during the later stages of an infection, acts as an inhibitor of viral replication, and is required for assembly of viral particles. (Sato *et al.*, 2004) Thus RNA editing by the cellular enzymes is critical to the virus' life cycle because it regulates the balance between viral replication and virion assembly. The virus contain a hypothetical protein (a protein that not prove experimentally) using protein sequence.

The HDV envelope protein has three of the HBV surface proteins anchored to it. The S region of the genome is most commonly expressed and its main function is to assemble subviral particles. HDV antigen proteins can combine with the viral genome to form a ribonucleoprotein (RNP) which when enveloped with the subviral particles can form viral-like particles that are almost identical to mature HDV, but they are not infectious. Researchers had concluded that the determinant of infectivity of HDV was within the N-terminal pre-S1 domain of the large protein (L). It was found to be a mediator in binding to the cellular receptor. Recently, researchers Georges AbouJaoudé and Camille Sureau published an article that studied the role of the antigenic loop, found in HDV envelope proteins, in the infectivity of the virus. The antigenic loop, like the N-terminal pre-S1 domain of the large protein, is exposed at the virion surface. Jaoudé and Sureau's study provided evidence that the antigenic loop may be an important factor in HDV entry into the host cell and by mutating parts of the antigenic loop, the infectivity of HDV may be minimized. (Radjef *et al.*, 2004).

In this study we use a bioinformatics tools to develop a vaccine that can prevent infection of HDV in future by identifying epitopes from T and B cells. The study covers the HDAg protein and a hypothetical **protein in the virus.**

MATERIAL AND METHODS

Sequence retrieval

The sequences of the two antigenic protein HDAg-L 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 Hepatitis Delta 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 ABCpredhttp:// crdd.osdd. net/raghava/abcpred/) (Saha and Raghava, 2006) andBCpred for conformation result. In ABCpredmethod 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 come 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 M,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/)(PonomarenkoJV,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 confirmation of the immunogenic character of all epitopes fragments Vaxijen 2.0 server (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) (Irini A Doytchinova and Darren R 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 allerginecity of selected epitopes and their conscious variants was predicted using AllerTop server (https://www.ddg-pharmfac.net/AllerTOP/). ALLerTOP uses the auto cross covariance (ACC) method. The server is trained on several known allergies and non-allergens from different species.

For toxicity using ToxinPred(http://crdd.osdd.net/raghava/toxinpred/) (SudheerGupta *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. (J Yang, R Yan, et al, 2015). For docking ClusPro sever (https://cluspro.bu.edu/login.php) (DimaKozakov, 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

Immunodominant epitopes which can generate both antibody and cell mediated immunity were identified to generate memory cells against hepatitis D virus. We first predicted B and T cell epitopes and their possible MHC alleles from the hepatitis D virus for HDAg-L and hypothetical protein using the tools mentioned in the methods

Identification and prediction of B-cell epitopes

In this study HDAg protein was used. B-cell epitopes the BCEpred server which show 5 epitopes with high conservancy in the sequence to generate immune response (SRPEGRKNRGGREEV, LGKKDKDGEGAPPAK RARTD QMEVD SGPRKRPSRGG, TDKERQDHRRRKA, KNLSKEEEEELRRLTEEDERRERR, and EGGTRGA). Also for more confirmation to B-cell epitopes and it conservancy the ABCpred server was used, the results were shown in 21 epitopes with high conservancy and their score was up to 0.5. Table (1) showed the results of ABCpred. The conservancies in the two software were measured using IEDB conservation tools that predicted both results of ABCpred and BCpred epitopes and were 100% conserved and can generate immune response.

Table 1: result of ABCpred and conservancy for HDAg-L protein.

Rank	Sequence	Start position	Score	Conservancy 100%
1	EDEHPWLGNI	45	0.87	C
2	LGNIKGILGK	51	0.8	C
3	GEGAPPAKRA	65	0.77	C
4	QDHRRRKALE	100	0.75	C
5	ERDLRKVKKK	31	0.72	C
5	KQLSAGGKNL	113	0.72	C
6	TDQMEVDSGP	76	0.7	C
6	KKIKKLEDEH	39	0.7	C
7	KALENKRKQL	106	0.69	C

8	RKRPSRGGFT	86	0.68	C
8	VSGRKKLEEL	21	0.68	C
9	SRGGFTDKER	90	0.67	C
10	RKNRGGREEV	7	0.65	C
10	LGKKDKDGEG	58	0.65	C
10	SRPEGRKNRG	2	0.65	C
11	FTDKERQDHR	94	0.64	C
12	KKLEELERDL	25	0.61	C
13	GGREEVLEQW	11	0.58	C
14	PPAKRARTDQ	69	0.56	C
14	RKVKKKIKKL	35	0.56	C
15	AGGKNLSKEE	117	0.51	C

For hypothetical protein the BCEpred showed 10 epitopes (KGSSGGDFS, PPGPSDREAR,GRQGQQSPALCSLKGSSGGD, GTLNPSGR, TWPPGPSDRE, LELGRQGQQ, QTWPPGPSDREARLL, RYGEIHP, ELGRQGQQSPLYRKE) and the ABCpred showd 11 epitopes (MVYSLSRCAL, ARLLLFHLPS, LWGTLNPSGR, FHLPSFPFLE, RYGEIHPLLA, ALCSLKGSSG, FSVLLRVASS, EFHWRYGEIH, HPLLAFSFFL, KGSSGGDFSV, QTWPPGPSDR) the epitopes from the two software showed 100% conservancy table (2) showed the result of ABCpred.

Table (2): result of ABCpred and conservancy for hypothetical protein

Rank	Sequence	Start position	Score	conservancy
1	MVYSLSRCAL	1	0.7	C
2	ARLLLFHLPS	55	0.69	C
3	LWGTLNPSGR	31	0.68	C
4	FHLPSFPFLE	60	0.67	C
5	RYGEIHPLLA	73	0.66	C
6	ALCSLKGSSG	9	0.65	C
7	FSVLLRVASS	21	0.64	C
8	EFHWRYGEIH	69	0.63	C
9	HPLLAFSFFL	78	0.58	
9	KGSSGGDFSV	14	0.58	C
10	QTWPPGPSDR	44	0.52	C

Identification and prediction of T-cell epitopes

For HDAg protein to form epitopes of T-cell MHC 1 the total of 16 epitopes for HLA-A, B and C by using NetMHC 4.0 with stronger binding rank 0.5% also with high conservancy was used. Table(3)showed the results of MHC II using ProPred server. A total of 9 selected epitopes after removing duplicates, with conservancy and threshold 3% and top score of 5 were showed in table (4).

Table 4: Result of MHC I for HDAg-L protein.

allele	epitopes	affinity	Rank
HLA-A0250	KLEDEHPWL	6.79	0.1
	WLGNIKGIL	20.42	0.3
HLA-A3001	RARTDQMEV	0.706	0.17
HLA-A3207	RKRPSRGG	0.656	0.1
	KKLEDEHPW	0.648	0.1
HLA-B0801	ENKRKQLSA	0.586	0.25
HLA-B0802	QDHRRRKAL	0.135	0.5
HLA-B0803	QMEVDSGPR		0.211
HLA-B1402	QDHRRRKAL	0.488	0.01
	AKRARTDQM	0.373	0.15
	TRGAPGGGF	0.478	
	QLSAGGKNL	0.418	0.3
HLA-B1503	MQGVPESPF	0.855	0.03
	RKRPSRGGF	0.785	0.15
HLA-B2720	PQVGGVNPL	0.595	0.25
	RERRIAGPQ	0.576	0.3

Table (5): Result of MHC II for HDAg-L protein.

Allele	Epitopes	Conservancy
DRB1_0102	LGNIKGILG	C
DRB1_0301	LERDLRKVK	C
	WLGNIKGIL	C
DRB1_0306	LRKVKKKIK	C
DRB1_0307	LSAGGKNLS	C
DRB1_0309	WVSGRKKLE	C
DRB1_0311	LRKVKKKIK	C
	LSAGGKNLS	C
DRB1_0801	VKKKIKKLE	C

For hypothetical protein 15 epitopes were predicted to cause immune in MHC I (HLA-A,B and C). Table (5) showed the results of 10 epitopes in MHC II. Table (6)showed the result the two software. Epitopes were 100% conserved.

Table (5): Results of MHC I for hypothetical protein.

Allele	Epitope	affinity	Rank
HLA-A0101	FLEFHWRY	0.469	0.3
HLA-A0202	LLAFSFFL	0.848	0.07
HLA-A0207	HLPSFPFL	0.116	0.8
HLA-A2403	RYGEIHPL	0.857	0.05
HLA-A2501	EIHPLLAF	0.089	1.6
HLA-A0207	HLPSFPFL	.0.457	.0.30
HLA-C0501	ISDVPMLF	0.352	0.4
HLA-C0702	FHLPSFPF	0.783	0.04
HLA-B0702	NPSGRSVL	0.522	0.5

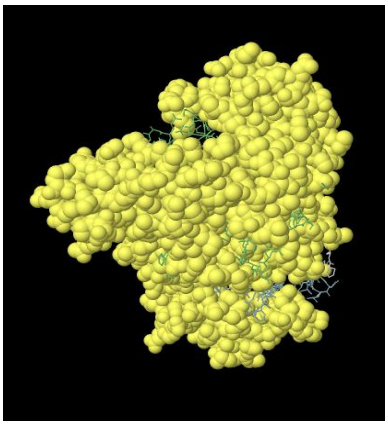
HLA-B0801	YSLSRCAL	0.775	0.02
HLA-B2705	ARLLLFHL	0.623	0.01
HLA-B3503	HPLLAFSF	0.382	0.08
HLA-B4801	RQGQQSPL	0.343	0.03
HLA-B5101	FPFLEFHW	0.372	0.17
HLA-B7301	WRYGEIHP	0.522	0.5

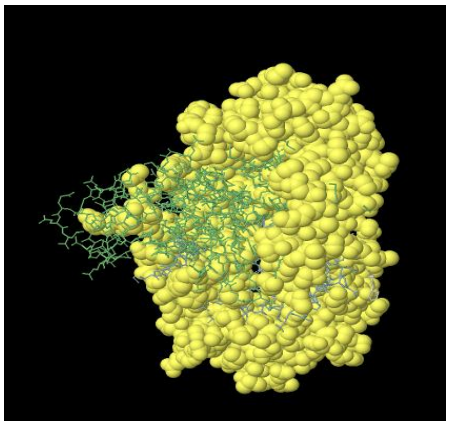
Table (6): Result of MHC II for hypothetical protein.

Allele	Epitope	Conservancy
DRB1_0101	MVYSLSRCA	C
DRB1_0102	LRVASSLWG	C
	LFHLPSFPF	C
DRB1_0305	FSVLLRVAS	C
DRB1_0307	MVYSLSRCA	C
DRB1_0401	MVYSLSRCALC	C
DRB1_0801	VYSLSRCAL	C
	VLLRVASSL	C
DRB1_1128	LLLFHLPSF	C
DRB5_0105	WGTLNPSGR	C

Antibody Epitope prediction

The prediction of antibody epitopes showed 26 linear epitopes and 5 discontinuous ones using Ellipro tools. Table (7) and (8) respectively showed the results for hepatitis delta virus HDAg-Lprotein (5M5V). Figure (1)and (2) showed the 3D structure of the residues.





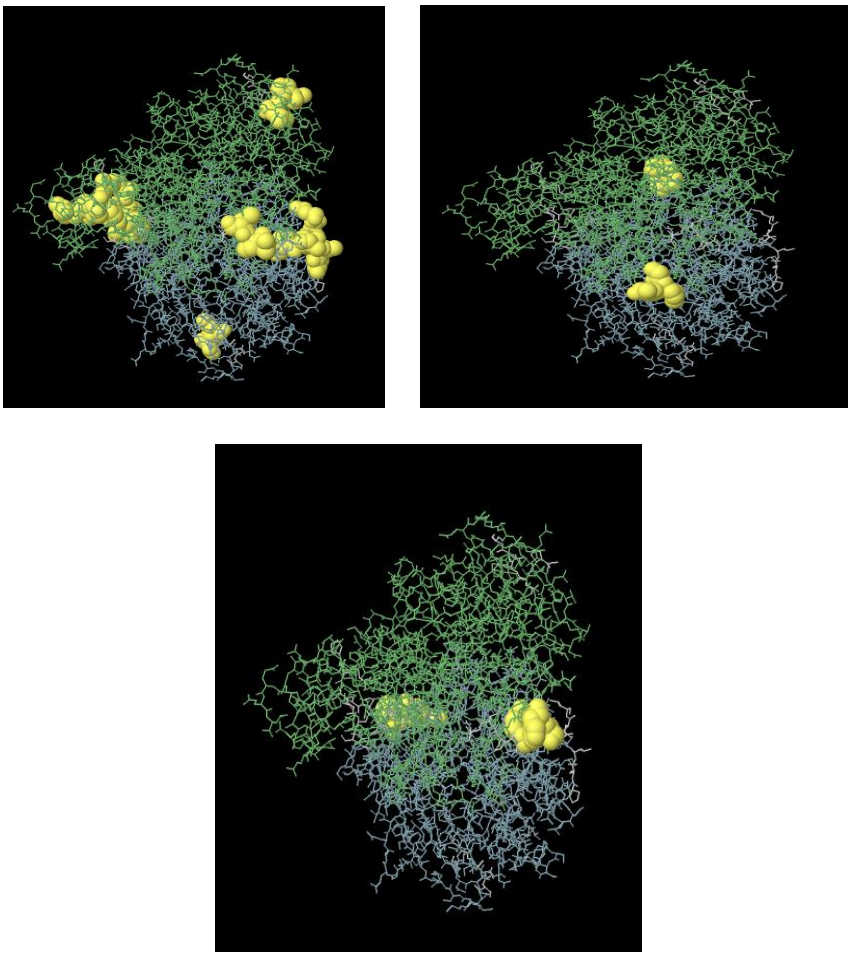
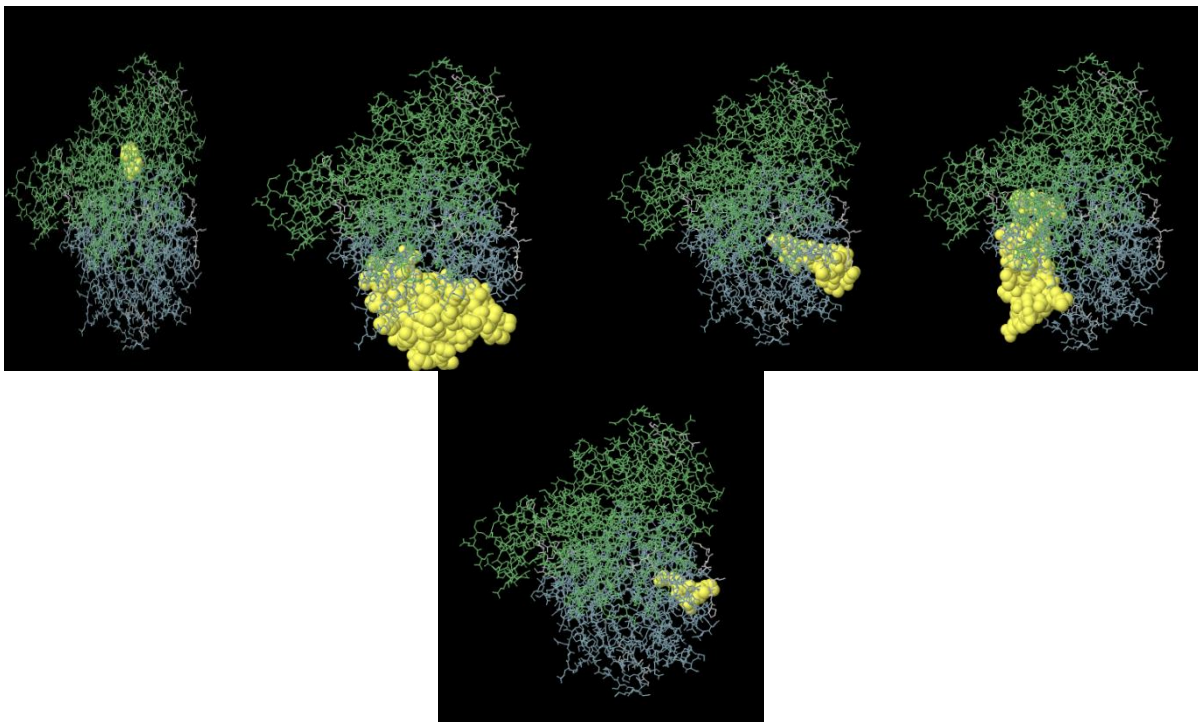


Figure (2): Result of discontinuous Epitope structure.



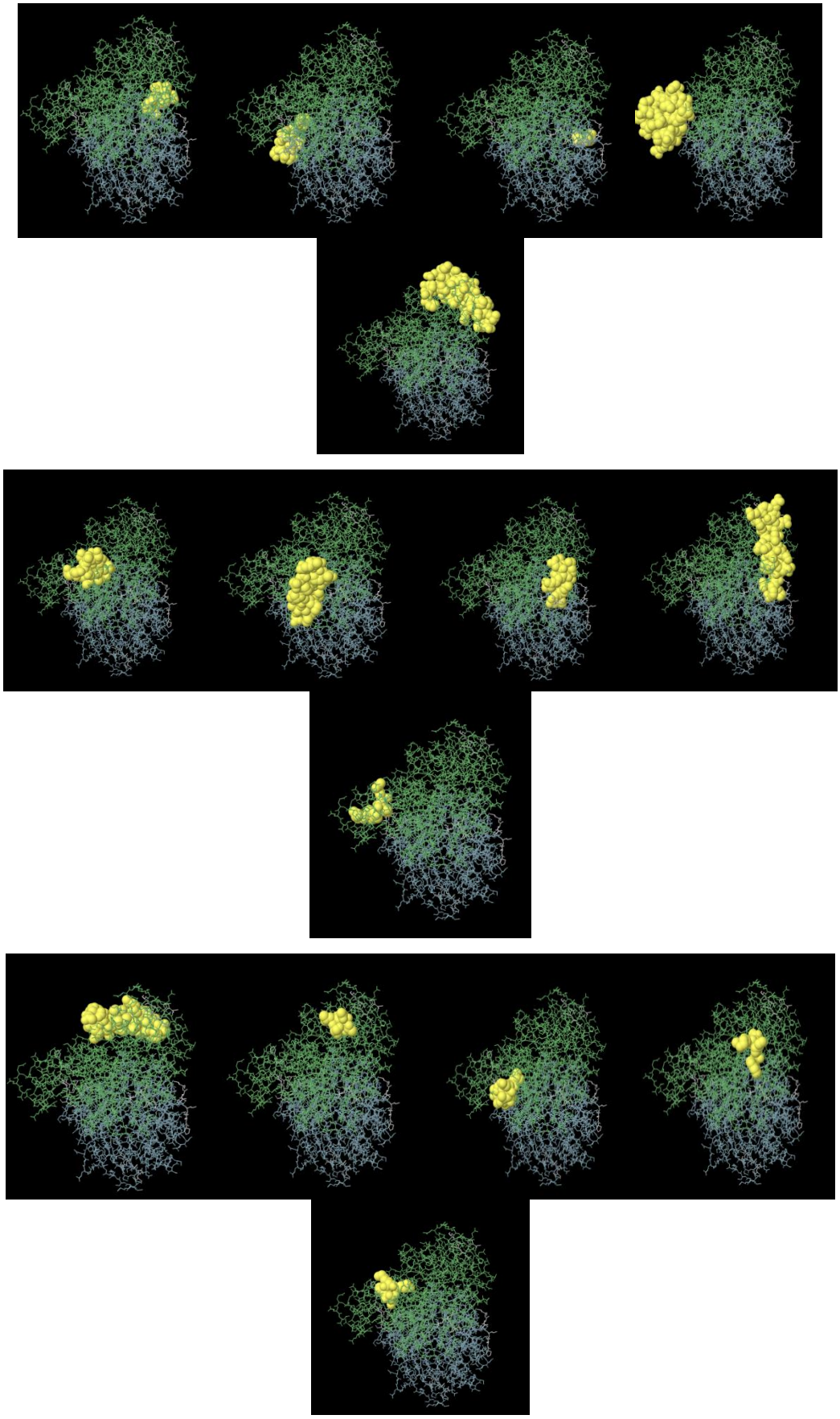


Figure (3): Result of discontinuous epitope structure.

Table (7): Result of linear Epitope.

19	B	268	271	EKHD	4	0.56
20	B	115	118	SLNT	4	0.524
21	B	222	225	GQAG	4	0.525
22	E	4	9	LPLLES	6	0.919
23	F	4	9	LPLLES	6	0.893
24	G	2	7	PRLPLL	6	0.625
25	H	2	5	PRLP	4	0.503
26	J	4	7	LPLL	4	0.815

Table (8): Result of discontinuous Epitope.

No.	Residues	Number of residues	Score
1	B:I4, B:L5, B:P6, B:R8, B:V44, B:G45, B:E46, B:Q47, B:A48, B:Q49, B:V50, B:I52, B:D54, B:M55, B:N56, B:D57, B:P58, B:S59, B:N60, B:P61, B:I62, B:R63, B:R64, B:P65, B:I66, B:S67, B:A68, B:D69, B:N74, B:P75, B:A76, B:S77, B:K78, B:V79, B:I80, B:L82, B:A84, B:G85, B:K86, B:T87, B:L88, B:Q89, B:I90, B:F91, B:N92, B:I93, B:E94, B:M95, B:K96, B:S97, B:K98, B:M99, B:K100, B:A101, B:H102, B:T103, B:M104, B:T105, B:D106, B:D107, B:I114, B:S115, B:L116, B:N117, B:T118, B:T123, B:D124, B:N125, B:A126, B:V127, B:Y128, B:H129, B:W130, B:S131, B:M132, B:E133, B:G134, B:E135, B:S136, B:Q137, B:P138, B:V139, B:K140, B:M141, B:F142, B:D143, B:R144, B:D159, B:A160, B:K161, B:Q162, B:K163, B:W164, B:L183, B:Y184, B:S185, B:V186, B:D187, B:R188, B:K189, B:V190, B:S191, B:Q192, B:P193, B:Q203, B:F204, B:K205, B:M206, B:E207, B:G208, B:N209, B:A210, B:E211, B:E212, B:S213, B:T214, B:L215, B:G222, B:Q223, B:A224, B:G225, B:K227, B:H229, B:I230, B:I231, B:E232, B:V233, B:G234, B:T235, B:P236, B:P237, B:T238, B:G239, B:N240, B:Q241, B:P242, B:F243, B:P244, B:K245, B:K246, B:A247, B:V248, B:D249, B:K269, B:H270, B:D271, B:D286, B:L287, B:E288, B:T289, B:G290, B:T291, B:C292, B:I293, B:V329, B:E330, B:E331, B:E332, B:N333, B:I334, B:I335, B:P336, B:Y337, B:I338, B:T339, B:N340, B:V341, B:L342, B:Q343, B:N344, B:P345, B:D346, B:L347, B:A348, B:L349, B:R350, B:M351, B:A352, B:V353, B:R354, B:N355, B:N356, B:L357, B:A358, B:G359, B:A360, B:E361, B:E362, B:L363, F:L4, F:P5, F:L6, F:L7, F:E8, F:S9, J:L4, J:P5, J:L6, J:L7	199	0.743
2	A:E11, A:H12, A:L13, A:Q14, A:L15, A:Q16, A:N17, A:L18, A:G19, A:I20, A:N21, A:P22, A:A23, A:N24, A:I25, A:G26, A:F27, A:S28, A:T29, A:D35, A:K36, A:F37, A:I38, A:C39, A:I40, A:R41, A:E42, A:K43, A:V44, A:G45, A:E46, A:Q47, A:A48, A:Q49, A:V50, A:V51, A:I52, A:I53, A:D54, A:M55, A:N56, A:D57, A:P58, A:S59, A:N60, A:P61, A:I62, A:R63, A:R64, A:P65, A:I66, A:S67, A:A68, A:D69, A:S70, A:A71, A:M73, A:N74, A:P75, A:A76, A:S77, A:K78, A:V79, A:I80, A:A81, A:L82, A:K83, A:A84, A:G85, A:K86, A:T87, A:L88, A:Q89, A:I90, A:F91, A:N92, A:I93, A:E94, A:M95, A:K96, A:S97, A:K98, A:M99, A:K100, A:A101, A:H102, A:T103, A:M104, A:T105, A:D106, A:D107, A:V108, A:T109, A:S115, A:L116, A:N117, A:V119, A:L121, A:V122, A:T123, A:D124, A:N125, A:A126, A:V127, A:Y128, A:H129, A:W130, A:S131, A:M132, A:E133, A:G134, A:E135, A:S136, A:Q137, A:P138, A:V139, A:K140, A:M141, A:F142, A:D143, A:R144, A:H145, A:S146, A:S147, A:L148, A:A149, A:G150, A:C151, A:K163, A:S171, A:A172, A:Q173, A:Q174, A:R176, A:V177, A:V178, A:Y184, A:S185, A:V186, A:D187, A:R188, A:V190, A:S191, A:F251, A:F252, A:P253, A:P254, A:E255, A:A256, A:Q257, A:N258, A:D259, A:F260, A:K278, A:Y279, A:S299, A:G300, A:E301, A:T302, A:N319, A:R320, A:K321, A:G322, A:Q323, E:L4, E:P5, E:L6, E:L7, E:E8, E:S9	170	0.716
3	G:R3, G:L4, G:P5, G:L6, G:L7	5	0.604
4	A:G222, A:Q223, A:A224, A:G225	4	0.516
5	B:H145, B:S146, B:S147, B:A149, B:G150, B:C151	6	0.508

Antigenicity, allerginecity and toxicity of Epitopes

The vaxijen server showed that all epitopes are antigen in nature for HDAg-L and hypothetical protein which can generate antibody. AllerTOP server showed no allergy cause from the two proteins. Toxinpred showed no toxic effect of the proteins.

3D structure and Docking

Using I-tasser a 3D structure for HDAg-L and hypothetical protein was visualized. UsingClusProto dock HDAg-L proteinwith its receptor in T-cell

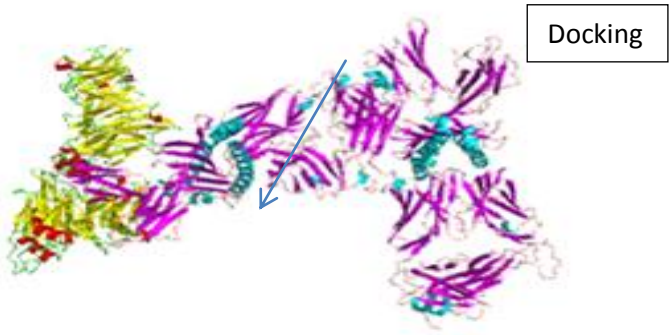


Figure (3): Showing the result of docking HDAg-L protein.

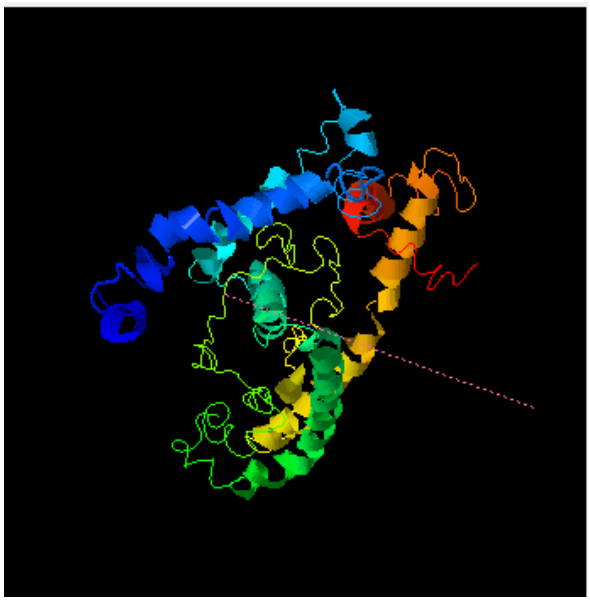


Figure (4): Result of 3D structure of HDAg-L protein.

DISCUSSION

As vaccines became an effective way to improve public health by building up adaptive immunity they still took long time to be prepared by the old habitual methods. In this study using bioinformatics tools and vaccine design to find the best epitopes which generate both humeral and cellular immune responses was the best way to boost the process and produce the vaccine within a limited time and effort. Also this method tested its potency and effectiveness in a shorter time. In this study the epitopes responsible for generating the immunity against Hepatitis Delta Virus in two proteins HDAg and hypothetical using molecular sequence in all software were identified to determine how the immune responses were elicited for hepatitis delta virus. The HDAg –L protein is mostly common protein known as antigen of hepatitis D virus and the rest of protein is unknown, but this study focus on the two proteins HDAg and the hypothetical protein. This was the first study to deal with the hypothetical protein as an antigen using bioinformnatics tools. The results showed us how can this two protein become antigenic and produce immune response. This study is novel because no research focus on hepatitis delta virus because it's not a main virus to detect but researches showed that Hepatitis D virus is very important due to it's a companying (co-infected) B virus and lead to a series pathogenic problems in the liver. Also in this study showed how validation and the binding affinity of the virus with receptors to generate immune response. Despite that the virus is RNA genetic composition but till now it doesn't showed a genetic drift which is good to design a vaccine from epitopes in this study. Most studies focus on hepatitis B virus due to its virulent effect and invasive infection to the liver

which may lead to cancer but some studies design pitopesforall hepatitis virus using revresvaccinol. (https://www.sciencedirect. com/science/article/abs/pii/S1567134820302197?dgcid=rss_sd_all) or study specific allele for the virus (https://www.microbiologyresearch. org/ content/journal/jgv/10.1099/vir.0.80183-0) or a wet-lab procedure without involve in bioinformatics tools epitopes design.

Recommendation

This study recommend that to apply this epitopes based vaccine, more wet-lab test to see and make more conformational tests.

REFERENCES

1. "Hepatitis (Viral) NIDDK". The National Institute of Diabetes and Digestive and Kidney Diseases. Retrieved 2020-06-19.
2. Farci P. "Delta hepatitis: an update". Journal of Hepatology, 2003; 39(1): S212–9.
3. Magnius L, Taylor J, Mason WS, Sureau C, Dény P, Norder H. "ICTV Virus Taxonomy Profile: Deltavirus". The Journal of General Virology, December, 2018; 99(12): 1565–1566. doi:10.1099/jgv.0.001150. PMID 30311870.
4. Makino S, Chang MF, Shieh CK, Kamahora T, Vannier DM, Govindarajan S, Lai MM. "Molecular cloning and sequencing of a human hepatitis delta (delta) virus RNA". Nature, 1987; 329(6137): 343–6. Bibcode:1987Natur.329..343M. doi:10.1038/329343a0. PMID 3627276.S2CID 4368061.
5. "Hepatitis D".www.who.int. Retrieved 2020-09-20
6. Fattovich G, Giustina G, Christensen E, Pantalena M, Zagni I, Realdi G, Schalm SW. "Influence of hepatitis delta virus infection on morbidity and mortality in compensated cirrhosis type B. The European Concerted Action on Viral Hepatitis (Eurohep)". Gut., March 2000; 46(3): 420–6. doi:10.1136/gut.46.3.420. PMC 1727859.PMID 10673308.
7. Poisson F, Roingeard P, Baillou A, Dubois F, Bonelli F, Calogero RA, Goudeau A. "Characterization of RNA-binding domains of hepatitis delta antigen".The Journal of General Virology, November 1993; 74(Pt 11): 2473–8. doi:10.1099/0022-1317-74-11-2473. PMID 8245865.
8. Zuccola HJ, Rozzelle JE, Lemon SM, Erickson BW, Hogle JM. "Structural basis of the oligomerization of hepatitis delta antigen".Structure, July 1998; 6(7): 821–30. doi:10.1016/S0969-2126(98)00084-7. PMID 9687364.

9. Elena SF, Dopazo J, Flores R, Diener TO, Moya A. "Phylogeny of viroids, viroidlike satellite RNAs, and the viroidlike domain of hepatitis delta virus RNA".Proceedings of the National Academy of Sciences of the United States of America, July 1991; 88(13): 5631–4. Bibcode:1991PNAS...88.5631E. doi:10.1073/pnas.88.13.5631. PMC 51931. PMID 1712103.
10. Sureau C. "The role of the HBV envelope proteins in the HDV replication cycle". Hepatitis Delta Virus. Current Topics in Microbiology and Immunology, 2006; 307: 113–31. doi:10.1007/3-540-29802-9_6. ISBN 978-3-540-29801-4.PMID 16903223.
11. Yan H, Zhong G, Xu G, He W, Jing Z, Gao Z, Huang Y, Qi Y, Peng B, Wang H, Fu L, Song M, Chen P, Gao W, Ren B, Sun Y, Cai T, Feng X, Sui J, Li W. "Sodium taurocholatecotransporting polypeptide is a functional receptor for human hepatitis B and D virus". eLife., November, 2012; 1: e00049. doi:10.7554/eLife.00049. PMC 3485615.PMID 23150796.
12. Engelke M, Mills K, Seitz S, Simon P, Gripon P, Schnölzer M, Urban S. "Characterization of a hepatitis B and hepatitis delta virus receptor binding site". Hepatology, April 2006; 43(4): 750–60. doi:10.1002/hep.21112. PMID 16557545.
13. Xia YP, Yeh CT, Ou JH, Lai MM. "Characterization of nuclear targeting signal of hepatitis delta antigen: nuclear transport as a protein complex". Journal of Virology, February 1992; 66(2): 914–21. doi:10.1128/JVI.66.2.914-921.1992. PMC 240792.PMID 1731113.
14. Lehmann E, Brueckner F, Cramer P. "Molecular basis of RNA-dependent RNA polymerase II activity". Nature, November 2007; 450(7168): 445–9. Bibcode:2007Natur.450..445L. doi:10.1038/nature06290. hdl:11858/00-001M-0000-0015-7EE1-9. PMID 18004386.S2CID 4393153.
15. Greco-Stewart VS, Schissel E, Pelchat M. "The hepatitis delta virus RNA genome interacts with the human RNA polymerases I and III". Virology, March, 2009; 386(1): 12–5. doi:10.1016/j.virol.2009.02.007. PMID 19246067.
16. Li YJ, Macnaughton T, Gao L, Lai MM. "RNA-templated replication of hepatitis delta virus: genomic and antigenomic RNAs associate with different nuclear bodies". Journal of Virology, July 2006; 80(13): 6478–86. doi:10.1128/JVI.02650-05. PMC 1488965.PMID 16775335.
17. Branch AD, Benenfeld BJ, Baroudy BM, Wells FV, Gerin JL, Robertson HD (February 1989). "An ultraviolet-sensitive RNA structural element in a viroid-like domain of the

hepatitis delta virus".Science. 243 (4891): 649–52. Bibcode: Sci., 1989; 243: 649B. doi:10.1126/science.2492676. PMID 2492676.

18. Weiner AJ, Choo QL, Wang KS, Govindarajan S, Redeker AG, Gerin JL, Houghton M). "A single antigenomic open reading frame of the hepatitis delta virus encodes the epitope(s) of both hepatitis delta antigen polypeptides p24 delta and p27 delta". Journal of Virology, February 1988; 62(2): 594–9. doi:10.1128/JVI.62.2.594-599.1988. PMC 250573.PMID 2447291.
19. 19-Sato S, Cornillez-Ty C, Lazinski DW. "By inhibiting replication, the large hepatitis delta antigen can indirectly regulate amber/W editing and its own expression". Journal of Virology, August 2004; 78(15): 8120–34. doi:10.1128/JVI.78.15.8120-8134.2004. PMC 446097.PMID 15254184.
20. Jaoudé GA, Sureau C. "Role of the antigenic loop of the atitis B virus envelope proteins in infectivity of hepatitis delta virus".Journal of Virology, August, 2005; 79(16): 10460–6. CiteSeerX 10.1.1.570.4147.doi:10.1128/jvi.79.16.10460-10466.2005. PMC 1182656.PMID 16051838.
21. Saha, S and Raghava G.P.S. Prediction of Continuous B-cell Epitopes in an Antigen Using Recurrent Neural Network. Proteins, 2006; 65(1): 40-48. PMID: 16894596
22. Saha.S and Raghava G.P.S. BcePred:Prediction of Continuous B-Cell Epitopes in Antigenic Sequences Using Physico-chemical Properties. In G.Nicosia, V.Cutello, P.J. Bentley and J.Timis (Eds.) ICARIS 2004, LNCS, 2004; 3239: 197-204, Springer.
23. Andreatta M, Nielsen MBioinformatics, Feb. 15, 2016; 32(4): 511-7, Gapped sequence alignment using artificial neural networks: application to the MHC class I system.
24. Nielsen M, Lundegaard C, Worning P, Lauemoller SL, Lamberth K, Buus S, Brunak S, Lund O.Protein Sci., Reliable prediction of T-cell epitopes using neural networks with novel sequence representations, 2003; 12: 1007-17.
25. Singh, H. and Raghava,G.P.S. ProPred: Prediction of HLA-DR binding sites. Bioinformatics, 2001; 17(12): 1236-37.
26. Ponomarenko JV, Bui H, Li W, Fusseder N, Bourne PE, Sette A, Peters B. ElliPro: a new structure-based tool for the prediction of antibody epitopes. BMC Bioinformatics, 2008; 9: 514. PMID: 19055730
27. Irini A Doytchinova and Darren R Flower. VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccines. BMC Bioinformatics, 2007; 8: 4.

28. Sudheer Gupta, PallaviKapoor, KumardeepChaudhary, AnkurGautam, Rahul Kumar, Open Source Drug Discovery Consortium,… Published: Septemb13, 2013 https://doi.org/10.1371/journal.pone.0073957
29. J Yang, R Yan, A Roy, D Xu, J Poisson, Y Zhang. The I-TASSER Suite: Protein structure and function prediction. Nature Methods, 2015; 12: 7-8. (PDF and supplementary).
30. DimaKozakova,b,d,*, David R. Hallc, Bing Xiab, Kathryn A. Porterb, DzmitryPadhornya, Christine Yuehb, Dmitri Beglovb, and SandorVajda, Nat Protoc, February, 2017; 12(2): 255–278. doi:10.1038/nprot.2016.169.