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Vaccine Design for Marburg Virus Using VP35 Protein

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Abstract: Background: Marburg virus is cause hemorrhagic fever for human, and transmits by exposure to one species of fruit bats, and transmits from person to person by body fluid it cause fever and bleeding but not like Ebola virus symptoms. Aim: in this study use bioinformatics tools to design a vaccine using vp35 protein which is part of virus cell that can help to prevent Marburg virus from spreading. Material and Method: after retrieval sequence of VP35 protein from National center for biotechnology information (NCBI) ABCpred were used to indicate B-cell epitopes and NetMHc to identify T-cell epitopes. To identify antibody prediction epitopes for linear and discontinuous IEDP Elipro tools and their 3D structure was used. To identify allerginicity Allertop server and vaxijen 2 servers were used for the toxicity and Toxinpred was used. Results: a 32 B-cell epitopes and 72 T-cell epitopes with high conservancy epitopes, and no allergy mentioned and toxicity, also the 3D structure represent the predicted epitope vaccine in the two way discontinuous and linear one. Conclusion: Marburg virus is a very limited outbreak virus but once it start to infect someone, it will start to spread powerfully to infect many, due to its process of infection plus its dangerous that no treatment yet and even no vaccine, some scientists link Marburg virus with Ebola because they have the same symptoms and the same way of infection and source which is the fruit bat, but still commonly different in many ways but the complications remain the same. This designed vaccine can help and prevent people specially where disease outbreak.

Keywords: NTCP, ABCpred, NetMHC, Ellipro

1. Introduction

Marburg virus disease (MVD) is a hemorrhagic fever and cause death up to 88% of people and is the same as Ebola virus, the disease first discover in three cases appear in Marburg and Frankfurt and one in Serbia Belgrade [1]. This outbreak happen when a laboratory work on African green monkey *Cercopithecus aethiops* which imported from Uganda, also sporadic cases were reported in Angola, Congo, Kenya and south Africa. In 2008 two cases reported due to visited a cave in Uganda inhabited by rousettus bat. Lately discover that monkey was infected from African fruits bat *Rousettus aegyptiacus*, Which is reservoir host of the virus [1]. This Rousettus bat is a sighted cave dwelling bat widely distributed across Africa, more areas are potentially at risk for outbreaks of Marburg virus than previously suspected.

The virus transmit through person to person contact by skin or mucous membrane in the eyes, nose or mouth with blood or body fluids like urine, saliva sweat, feces..etc. and by object contaminated with body fluid from a person who is sick with or has died from Marburg virus disease such as clothes, bedding, needles and medical equipment, Also semen from a man who recovered from the disease through vaginal or anal sex [1]. Spread of the virus between people has occurred in close environment and among direct contacts. Laboratory exposures can also occur when lab staff handle live Marburg virus [2].

The incubation period is 2 – 21 days after that the symptoms onset is sudden and marked fever, chills, headache and myalgia [3]. Around the fifth day a maculopopular rash most prominent in the trunk may occur symptoms. Nausea, vomiting, chest pain a sore throat, abdominal pain and

diarrhea may also appear, then become increasingly severe and can include jaundice, inflammation of the pancreas, severe weight loss, delirium, shock, liver failure, massive hemorrhagic and multi-organ dysfunction [4].

The diagnosis of the Marburg virus disease is difficult in the lab because many of the signs and symptoms are similar to other infected disease such as malaria, typhoid fever or Ebola. The diagnosis include antigen capture enzyme linked immunosorbent assay (ELISA) testing and polymerase chain reaction (PCR) and IgM capture ELISA can be used to confirm a case of the virus within a few days of symptoms onset [5].

The Marburg virus is following the filoviridae family which is subdivided into three genera, marburgvirus, Ebolavirus and Cuevavirus. The genus Marburgvirus include single species Marburg Marburgvirus which is represented by two distinct viruses Marburg virus (MARV) and Ravin virus (RAVV) [6].

The Marburg virus has non-segmented negative sense RNA genome and have seven proteins, the single surface protein glycoprotein (GP) is in membrane it require for attachment, receptor binding and fusion. And enhance budding [7]. The viral protein VP40 is a typical viral matrix protein and mediated budding in contrast to EBOV VP40 MARV VP40 antagonize the single transducer and activator of transcription singling pathway and plays role as a virulent factor in host adaptation. The helical MARV ribonucloprotein complex or nucleocapsid is composed of 5 viral proteins, nucleoprotein (NP), VP35, VP30, large protein (L) and the viral RNA. The VP24 is protein unique to filoviruese is loosely attached to the nucleocapsid its involved in viral particle release, possibly in nucleocapid maturation, and might also play a role in the regulation of viral genome replication. The VP35 is multifunctional protein involved in nucleocapsid formation, viral RNA synthesis and the suppression of antiviral responses [8]. The VP30 protein function in the replication cycle but the mechanism is poorly understood. But its tightly associated with the nucleocapsid complex it's dispensable for proper nucleocapsid formation. The VP30 has moderate effect on gene expression, but is still essential for viral replication [9]. Figure 1 show detail structure of the Marburg virus and its proteins contents. (*https://viralzone.expasy.org/224?outline=all_by_species*)

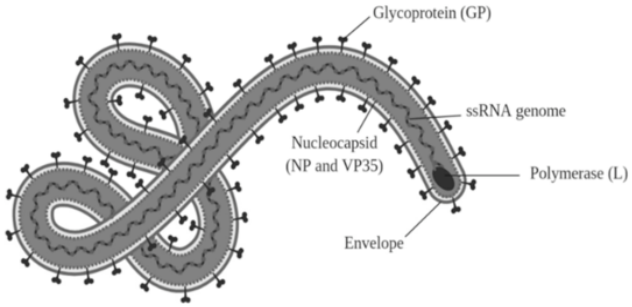


Figure 1. *Show structure and protein of Marburg virus.*

In this study the VP35 protein (PDB ID 5XSQ) is use to design a vaccine and generate antibody, the viral enter the body cell through endocytosis process and fusion and this mediated by glycoprotein where it bind to different type of C-type lectins or TAM receptor protein kinases [10]. The vaccine is designed using bioinformatics tools which are become booming nowadays. And help scientist to develop and avoid complications of wet lab. Figure 2 show the entering of the Marburg virus through cell membrane.

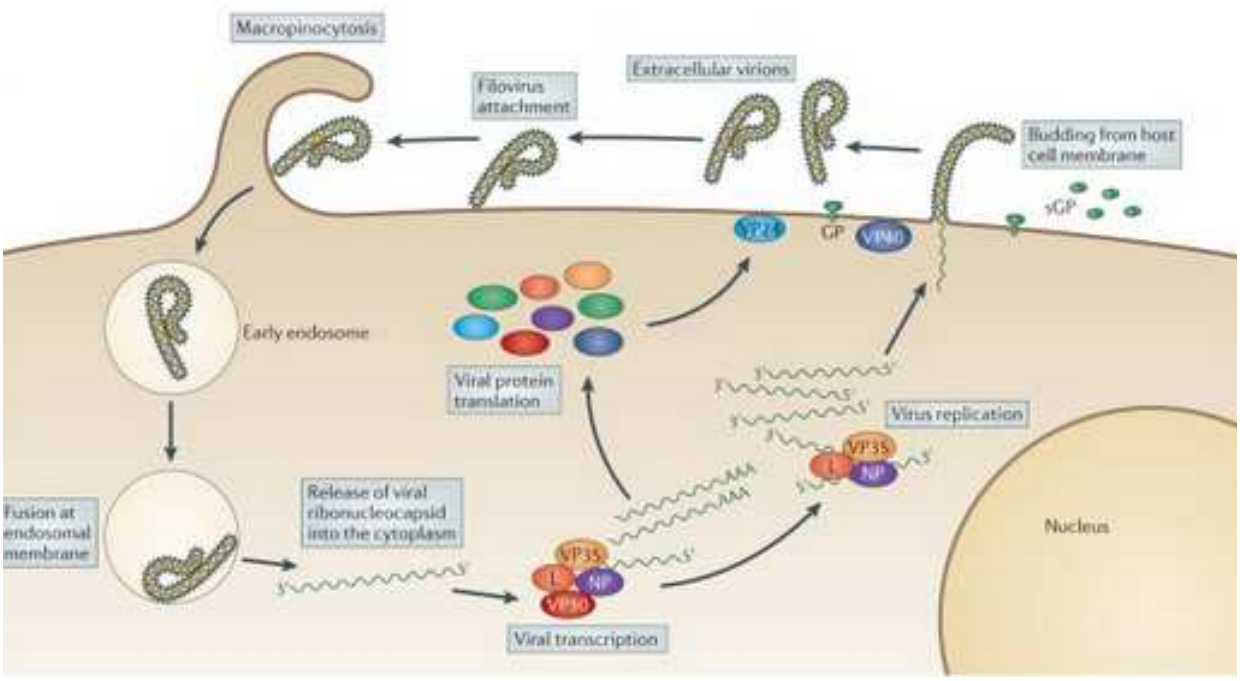


Figure 2. *Show entering of Marburg virus into body cell (ilhem M, et al 2015).*

2. Material and Method

2.1. Sequence Retrieval

The sequence of VP35 protein was obtained from the national center of biotechnology information (NCBI) (https://www.ncbi.nlm.nih.gov/). The sequence will be applied in different software to select the epitopes that can generate human immune response against the T and B cells. The predicted epitopes will be subjected to IEDB Ellipro for antibody epitope prediction. The vaxigen software was also use to predict good vaccine design. Allertop software use to predict allerginicity against the vaccine. Toxinpred is software use to predict toxicity of the vaccine. The conservancy of the predicted vaccine was also checked scoring >100%. The selection and prediction of the epitopes depend on both cellular and humeral immune response that B and T cell response.

2.2. Identification and Predictions of B Cell Epitopes

In the Linear B cell software was applied server the ABCpred (http://crdd.osdd.net/raghava/abcpred/) [11]. This software based on artificial neural network, it relies on random peptides trained on similar B cell epitope positive data. Selection of windows length 10 results comes out on graphical or tabular frame. Adjusting the threshold into +3 to -3 is more threshold lead to better specify with less sensitivity.

2.3. Identification and Predictions of T Cell Epitopes

Using NetMHC 4.0 (http://www.cbs.dtu.dk/services/NetMHC/) [12]. 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 it also use ANN method for predictions [13].

2.4. Conservancy Prediction

To assure that these epitopes of B cell and T cell are conserve we apply the epitopes of the two cell into conservation tools of the IEDB (http:// tools.iedb.org/ conservancy/) and this for linear and discontinuous sequence with threshold conservancy >100% this help in best selected epitopes.

2.5. Antibody Epitope Prediction

Using IEDB Ellipro (http://tools.iedb.org/ellipro/) [14]. to predict linear and conformational B cell by using VP35 protein Ellipro predict epitope with a score defined as PI (protrusion index) value averaged over epitope residues. In the method the protein 3D shape is approximately by a number of ellipsoids. Thus that the ellipsoid with PI =0.9 would include within 90% of the protein residues being outside of the ellipsoid; while the ellipsoid with PI=0.8 would include within 90% of the protein residues with 10% of the protein residues being outside the Ellipsoid while the ellipsoid with PI=0.8 would include 80% of residues with 20% being outside the Elliposoid. For each residue a PI value is defined based on the residues center of mass lying outside the largest possible ellipsoid for example all residues that are outside the 90% ellipsoid will have score 0.9. Residues with larger scores are associated with greater solvent accessibility discontinuous epitopes are defined based on PI value and are clustered based on the distance R (in A between residues centers of mass). The larger R is associated with the larger discontinuous epitopes being predicted.

2.6. Antigenicity, Allergencity and Toxicity

To confirm that immunogenic character of all epitopes fragments Vaxijen 2.0 server (http://www.ddg pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) [15]. it's on the alignment independence method which predict antigenicity using physiochemical properties and ACC methods for antigenicity assessments peptide fragment with a threshold greater than 0.4 were marked as potentially antigenic.

For allergenicity of selected epitopes and their conscious variant 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 allergents from different species.

For toxicity using Toxinpred (http://crdd.osdd.net/raghava/toxinpred/) [16]. is a web server which applies machine learning approaches using different properties of the peptides.

3. Result

3.1. Identification and Prediction of B Cell Epitopes

The ABCpred of B cell proposed 32 epitopes, and all epitopes are 100% conserve using IEDB conservation tools and can generate immune response table 1 show the result of ABCpred.

Table 1. *Show result of ABCpred.*

Rank	Epitope	Start position	Score	Conservancy 100%
1	GRTTAPAAAFDAYLNE	139	0.95	C
2	VGLQCSPCLMSKATST	43	0.92	C
3	AQQACSKGTMVKNETT	171	0.9	C
4	NETTDAADKMSKVLEL	183	0.88	C
4	LMTGKVPIDQVFGANP	14	0.88	C
5	AALTRLSRTFDAFLGV	264	0.87	C

Rank	Epitope	Start position	Score	Conservancy 100%
6	CLMSKATSTDDIVWDQ	50	0.86	C
6	AFHQILSEGENAQAAL	251	0.86	C
7	YKRRKPKGTVGLQCSP	34	0.83	C
7	SKIAYKSGKSGAFLDA	236	0.83	C
8	LRAVPPNPTIDKGWVC	300	0.79	C
8	NEHGVPPPQPAIFKDL	153	0.79	C
8	KGMSEMLAKYDHLVIS	122	0.79	C
9	FQTVPRPCQKSLRAVP	289	0.78	C
9	ELSEETFSKPNLSAKD	197	0.78	C
10	VHEIERQLHEITPVLK	97	0.77	C
10	PFHILAQVLSKIAYKS	227	0.77	C
11	GWVCVYSSEQGETRAL	312	0.76	C
12	SYMQQVSEGLMTGKVP	5	0.74	C
13	NPTIDKGWVCVYSSEQ	306	0.73	C
14	NRQISDIQSTLNEVTT	80	0.72	C
15	NPLEKLYKRRKPKGTV	28	0.69	C
15	QPAIFKDLGVAQQACS	161	0.69	C
16	AFLGVVPPVIRVKNFQ	275	0.68	C
16	LHEITPVLKMGRTLEA	104	0.68	C
17	GKSGAFLDAFHQILSE	243	0.67	C
18	THLPGNNTPFHILAQV	219	0.66	C
19	DQLIVKKTLADLLIPI	64	0.63	C
19	TDDIVWDQLIVKKTLA	58	0.63	C
20	QSTLNEVTTRVHEIER	87	0.6	C
21	KDLALLLFTHLPGNNT	211	0.54	C

3.2. Identification and Prediction of T Cell Epitopes

Using NetMHC 4 server for MHC class I showed 72 epitopes for allele HLA-A, B and C. and all with 100% conservancy. Table 2 show result of NetMHC predicted T cell epitopes.

Table 2. *Show result of NetMHC.*

Allel	Epitope	Affinity	Rank	Conservancy 100%
HLA-A0101	TIDKGWVCVY	115.32	0.17	C
HLA-A0201	KTLADLLIPI	8.27	0.08	C
HLA-A0301	KLYKRRKPK	25	0.12	C
	AVLSKIAYK	25	0.17	C
	KLYKRRKPK	42.15	0.2	C
	FHILAQVLSK	86.51	0.4	C
	QVFGANPLEK	115.08	0.5	C
HLA-A2601	EAISKGMSE	38.95	0.5	C
	TIDKGWVCVY	1007.29	0.5	C
HLA-B0702	TPRPCQKSL	23.03	0.12	C
	RPCQKSLRAV	23.91	0.12	C
	TPVLKMGRTL	27.35	0.12	C
	APAAAFDAYL	48.14	0.25	C
	VPRPCQKSL	62.48	0.3	C
HLA-B0801	MLAKYDHLVI	140.33	0.4	C
	LIVKKTLADL	226.07	0.5	C
HLA-B4001	SEMLAKYDHL	70.14	0.25	C
	SEGENAQAAL	151.06	0.5	C
	SEETFSKPNL	173.87	0.5	C
HLA-B5801	KATSTDDIVW	8.86	0.05	C
HLA-B1501	RQISDIQSL	26.69	0.3	C
	AQQACSGTM	26.69	0.25	C
	LAQVLSKIAY	35.9	0.4	C
HLA-B0702	TVPRPCQKSL	23.03	0.12	C
	RPCQKSLRAV	23.91	0.12	C
	TPVLKMGRTL	27.35	0.12	C
	APAAAFDAYL	27.35	0.25	C
	VPRPCQKSL	62.48	0.3	C
HLA-B0801	MLAKYDHLVI	140.33	0.4	C
	LIVKKTLADL	226.07	0.5	C
HLA-B1501	RQISDIQSTL	26.69	0.25	C
	AQQACSKGTM	29.09	0.3	C
	LAQVLSKIAY	35.9	0.4	C

Allel	Epitope	Affinity	Rank	Conservancy 100%
HLA-B3501	TAPAAAFDAY	14.96	0.09	C
	LAQVLSKIAY	89.98	0.4	C
HLA-B3801	THLPGNNTPF	447.84	0.15	C
HLA-B4001	SEMLAKYDHL	70.14	0.25	C
	SEGENAQAAL	151.06	0.5	C
	SEETFSKPNL	173.87	0.5	C
HLA-B4002	HEIERQLHEI	90.46	90.46	C
	SEMLAKYDHL	111.21	0.4	C
HLA-B4201	TPFHILAQVL	51.53	0.06	C
	TVPRPCQKSL	81.64	0.1	C
	TPFHILAQV	240.6	0.3	C
	PPQPAIFKDL	305.68	0.4	C
	VPRPCQKSL	318.08	0.4	C
HLA-B4201	TPFHILAQVL	51.53	0.06	C
	TVPRPCQKSL	81.64	0.1	C
	TPFHILAQV	240.6	0.3	C
	PPQPAIFKDL	305.68	0.4	C
	VPRPCQKSL	318.08	0.4	C
HLA-B4402	SEMLAKYDHL	29.21	0.06	C
	SEGENAQAAL	342.26	0.5	C
	MSEMLAKYDH	354.74	0.5	C
HLA-B4403	SEMLAKYDHL	319.11	0.5	C
HLA-B4501	SEGENAQAA	195.99	0.4	C
	SEGENAQAA	293.65	0.5	C
HLA-B4801	RQLHEITPVL	1693.35	0.17	C
	RQISDIQSTL	3749.15	0.5	C
HLA-B5101	IPINRQISDI	712	0.17	C
	LPGNNTPFHI	824.45	0.17	C
	LRAVPPNPTI	2436.3	0.5	C
HLA-B5301	LPGNNTPFHI	31.08	0.07	C
HLA-B5401	NTPFHILAQV	131.01	0.4	C
	LPGNNTPFHI	141.41	0.4	C
	CSPCLMSKAT	254.51	0.5	C
HLA-B5701	KATSTDDIVW	43.48	0.15	C
	RTLEAISKGM	192.33	0.5	C
HLA-B5801	KATSTDDIVW	8.86	0.9	C
HLA-B5802	KATSTDDIVW	11257.88	0.3	C
HLA-C0802	FLDAFHQIL	2872.68	0.4	C
	FLDAFHQIL	2968.99	0.4	C

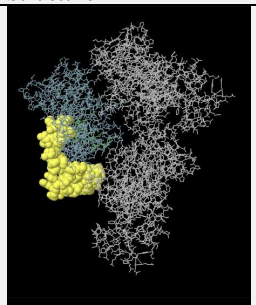
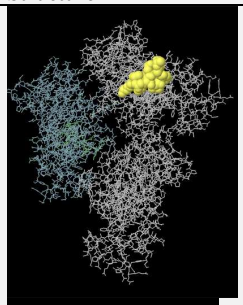
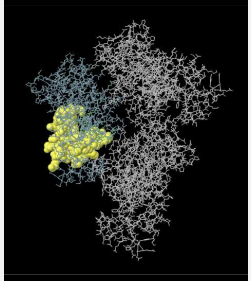
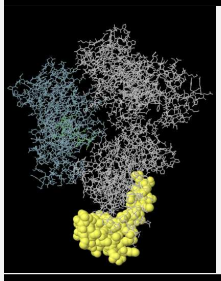
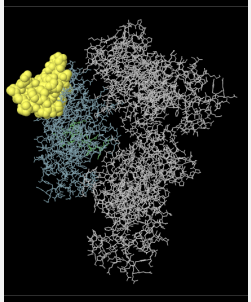
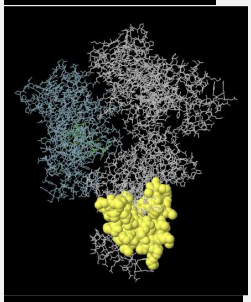
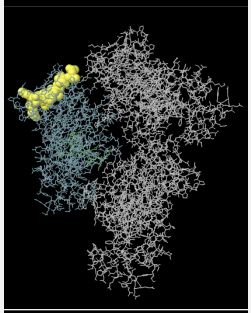
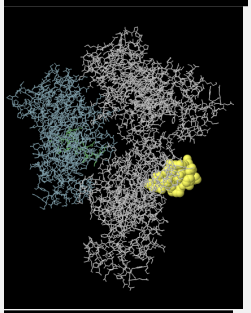
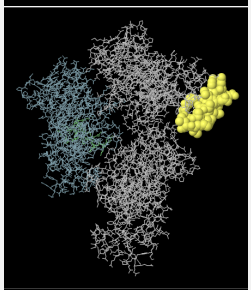
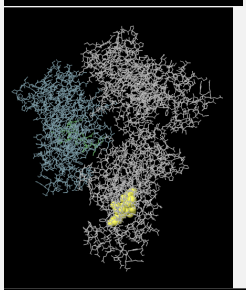
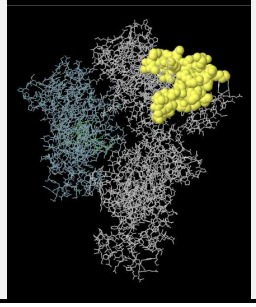
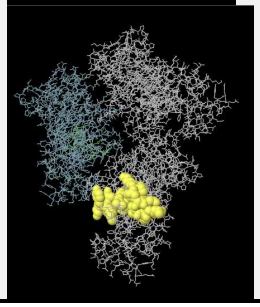
3.3. Antibody Epitope Prediction

Using Ellipro software from IEDB with PDB id (5XSQ) showed that 15 predicted linear epitopes table 3 show result of linear epitopes and 7 discontinuous epitopes, table 4 show result of discontinuous epitopes. Also it shows the 3D structure of both linear and discontinuous epitopes. Figures 1 and 2 show the 3D structure of linear and discontinues epitopes respectively.

Table 3. *Showing results of linear epitopes.*

No.	Chain	Start	End	Peptide	Number of residues	Score
1	A	264	320	RVLNLSGINNLEHGLYPQLSAIALGVATAHGSTLAGVNVGEQYQQLREAAHDAEVKL	57	0.822
2	A	209	257	GLLIVKTVLEFILQKTDSGVTLHPLVRTSKVKNEVASFKQALSNLARHG	49	0.808
3	A	63	103	KYLRDAGYEFDVIKNADATRFLDVIPNEPHYSPLILALKTL	41	0.684
4	A	5	13	KKVILFDTN	9	0.6
5	B	5	27	SYMQQVSEGLMTGKVPIDQVFGA	23	0.729
6	C	298	320	AGVNVGEQYQQLREAAHDAEVKL	23	0.747
7	C	220	256	ILQKTDSGVTLHPLVRTSKVKNEVASFKQALSNLARH	37	0.712
8	C	62	105	AKYLRDAGYEFDVIKNADATRFLDVIPNEPHYSPLILALKTLES	44	0.706
9	C	266	292	LNLSGINNLEHGLYPQLSAIALGVATA	27	0.658
10	C	5	13	KKVILFDTN	9	0.629
11	D	17	27	GKVPIDQVFGA	11	0.657
12	E	268	320	LSGINNLEHGLYPQLSAIALGVATAHGSTLAGVNVGEQYQQLREAAHDAEVKL	53	0.89
13	E	207	255	FSGLLIVKTVLEFILQKTDSGVTLHPLVRTSKVKNEVASFKQALSNLAR	49	0.814
14	E	87	102	IPNEPHYSPLILALKT	16	0.575
15	E	127	133	LVVGDRA	7	0.533
16	F	5	27	SYMQQVSEGLMTGKVPIDQVFGA	23	0.732

Table 4. *Showing results of linear epitopes and their 3D structure.*

NO	Peptide	Structure	NO	Peptide	Structure
1-	RVLNLSGINNLEHGLYP QLSAIALGVATAHGSTL AGVNVGEQYQQLREA AHDAEVKL		9-	LNLSGINNLEHGLYP QLSAIALGVATA	
2-	GLLIVKTVLEFILQKTD SGVTLHPLVRTSKVKNE VASFKQALSNLARHG		10-	KKVILFDTN	
3-	KYLRDAGYEFDVIKNA DATRFLDVIPNEPHYSP LILALKTL		11-	GKVPIDQVFGA	
4-	KKVILFDTN		12-	LSGINNLEHGLYPQLS AIALGVATAHGSTLA GVNVGEQYQQLREA AHDAEVKL	
5-	SYMQQVSEGLMTGKV PIDQVFGA		13-	FSGLLIVKTVLEFILQ KTDSGVTLHPLVRTS KVKNEVASFKQALSN LAR	
6-	AGVNVGEQYQQLREA AHDAEVKL		14-	IPNEPHYSPLILALKT	

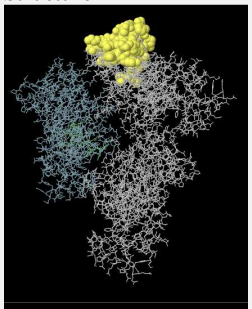
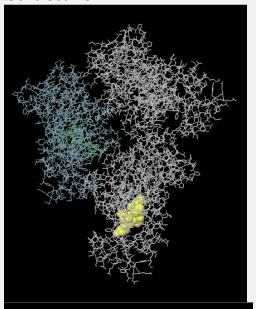
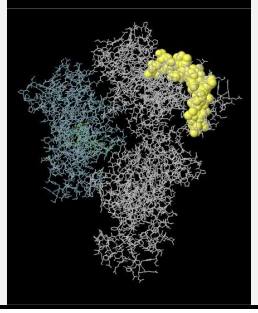
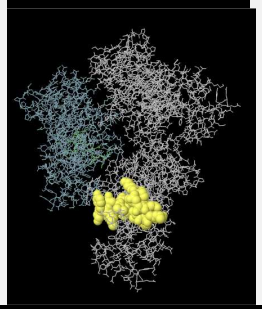
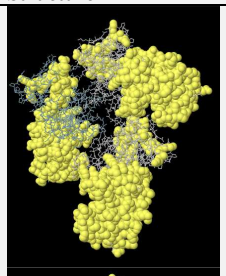
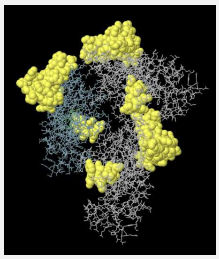
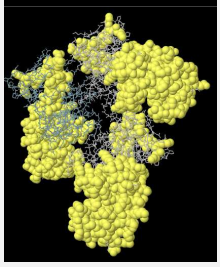
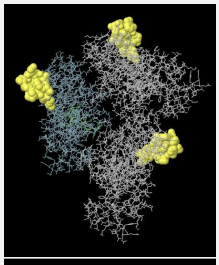
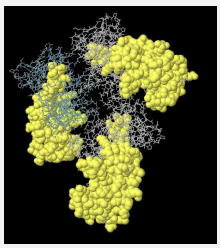
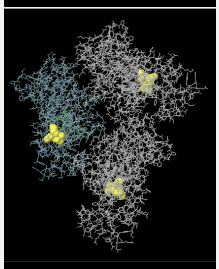
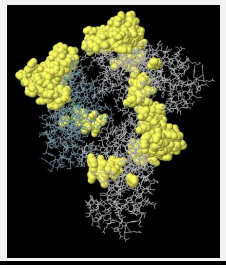
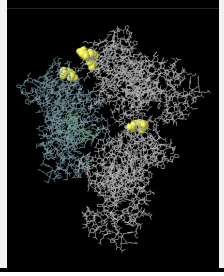
NO	Peptide	Structure	NO	Peptide	Structure
7-	ILQKTDSGVTLHPLVRT SKVKNEVASFKQALSN LARH		15-	LVVGDRA	
8-	AKYLRDAGYEFDVIKN ADATRFLDVIPNEPHYS PLILALKTLES		16-	SYMQQVSEGLMTGK VPIDQVFGA	

Table 5. *Showing results of Discontinuous epitopes.*

No.	Residues	Number of residues	Score
1	E:K126, E:L127, E:V128, E:V129, E:G130, E:D131, E:R132, E:A133, E:E136, E:F207, E:S208, E:G209, E:L210, E:L211, E:I212, E:V213, E:K214, E:T215, E:V216, E:L217, E:E218, E:F219, E:I220, E:L221, E:Q222, E:K223, E:T224, E:D225, E:S226, E:G227, E:V228, E:T229, E:L230, E:H231, E:P232, E:L233, E:V234, E:R235, E:T236, E:S237, E:K238, E:V239, E:K240, E:N241, E:E242, E:V243, E:A244, E:S245, E:F246, E:K247, E:Q248, E:A249, E:L250, E:S251, E:N252, E:L253, E:A254, E:R255, E:R264, E:L268, E:S269, E:G270, E:I271, E:N272, E:N273, E:L274, E:E275, E:H276, E:G277, E:L278, E:Y279, E:P280, E:Q281, E:L282, E:S283, E:A284, E:I285, E:A286, E:L287, E:G288, E:V289, E:A290, E:T291, E:A292, E:H293, E:G294, E:S295, E:T296, E:L297, E:A298, E:G299, E:V300, E:N301, E:V302, E:G303, E:E304, E:Q305, E:Y306, E:Q307, E:L309, E:R310, E:E311, E:A312, E:A313, E:H314, E:D315, E:A316, E:E317, E:V318, E:K319, E:L320, F:S5, F:Y6, F:M7, F:Q8, F:Q9, F:V10, F:S11, F:E12, F:G13, F:L14, F:M15, F:T16, F:G17, F:K18, F:V19, F:P20, F:I21, F:D22, F:Q23, F:V24, F:F25, F:G26, F:A27	134	0.808
2	A:F207, A:G209, A:L210, A:I212, A:V213, A:K214, A:T215, A:V216, A:L217, A:E218, A:F219, A:I220, A:L221, A:Q222, A:K223, A:T224, A:D225, A:S226, A:G227, A:V228, A:T229, A:L230, A:H231, A:P232, A:L233, A:V234, A:R235, A:T236, A:S237, A:K238, A:V239, A:K240, A:N241, A:E242, A:V243, A:A244, A:S245, A:F246, A:K247, A:Q248, A:A249, A:L250, A:S251, A:N252, A:L253, A:A254, A:R255, A:H256, A:G257, A:R264, A:L266, A:N267, A:L268, A:S269, A:G270, A:I271, A:N272, A:N273, A:L274, A:E275, A:H276, A:G277, A:L278, A:Y279, A:P280, A:Q281, A:L282, A:S283, A:A284, A:I285, A:A286, A:L287, A:G288, A:V289, A:A290, A:T291, A:A292, A:H293, A:G294, A:S295, A:T296, A:L297, A:A298, A:G299, A:V300, A:N301, A:V302, A:G303, A:E304, A:Q305, A:Y306, A:Q307, A:L309, A:R310, A:E311, A:A312, A:A313, A:H314, A:D315, A:A316, A:E317, A:V318, A:K319, A:L320, B:S5, B:Y6, B:M7, B:Q8, B:Q9, B:V10, B:S11, B:E12, B:G13, B:L14, B:M15, B:T16, B:G17, B:K18, B:V19, B:P20, B:I21, B:D22, B:Q23, B:V24, B:F25, B:G26, B:A27	127	0.801
3	C:V213, C:V216, C:I220, C:L221, C:Q222, C:K223, C:T224, C:D225, C:S226, C:G227, C:V228, C:T229, C:L230, C:H231, C:P232, C:L233, C:V234, C:R235, C:T236, C:S237, C:K238, C:V239, C:K240, C:N241, C:E242, C:V243, C:A244, C:S245, C:F246, C:K247, C:Q248, C:A249, C:L250, C:S251, C:N252, C:L253, C:A254, C:R255, C:H256, C:R264, C:L266, C:N267, C:L268, C:S269, C:G270, C:I271, C:N272, C:N273, C:L274, C:E275, C:H276, C:G277, C:L278, C:Y279, C:P280, C:Q281, C:L282, C:S283, C:A284, C:I285, C:A286, C:L287, C:G288, C:V289, C:T291, C:A292, C:G294, C:L297, C:A298, C:G299, C:V300, C:N301, C:V302, C:G303, C:E304, C:Q305, C:Y306, C:Q307, C:L309, C:R310, C:E311, C:A312, C:A313, C:H314, C:D315, C:A316, C:E317, C:V318, C:K319, C:L320, D:G17, D:K18, D:V19, D:P20, D:I21, D:D22, D:Q23, D:V24, D:F25, D:G26, D:A27	101	0.689
4	C:K5, C:K6, C:V7, C:I8, C:L9, C:F10, C:D11, C:T12, C:N13, C:A25, C:S28, C:G29, C:I30, C:D31, C:L32, C:G33, C:D34, C:L35, C:L36, C:E37, C:G38, C:N57, C:T58, C:A62, C:K63, C:Y64, C:L65, C:R66, C:D67, C:A68, C:G69, C:Y70, C:E71, C:F72, C:D73, C:V74, C:I75, C:K76, C:N77, C:A78, C:D79, C:A80, C:T81, C:R82, C:F83, C:L84, C:D85, C:V86, C:I87, C:P88, C:N89, C:E90, C:P91, C:H92, C:Y93, C:S94, C:P95, C:L96, C:I97, C:L98, C:A99, C:L100, C:K101, C:T102, C:L103, C:E104, C:S105	67	0.674
5	A:K5, A:K6, A:V7, A:I8, A:L9, A:F10, A:D11, A:T12, A:N13, A:I30, A:D31, A:L32, A:G33, A:D34, A:L35, A:L65, A:D67, A:A68, A:G69, A:Y70, A:E71, A:F72, A:D73, A:V74, A:I75, A:K76, A:N77, A:A78, A:D79, A:A80, A:T81, A:R82, A:F83, A:L84, A:D85, A:V86, A:I87, A:P88, A:N89, A:E90, A:P91, A:H92, A:Y93, A:S94, A:P95, A:L96, A:I97, A:L98, A:A99, A:L100, A:K101, A:T102, A:L103	53	0.667
6	E:I30, E:I87, E:P88, E:N89, E:E90, E:P91, E:H92, E:Y93, E:S94, E:P95, E:I97, E:L98, E:A99, E:K101, E:T102	15	0.582
7	C:D131, C:T160, C:G161, C:K164	4	0.542
8	C:S51, C:K53, C:D54	3	0.518

Table 6. *Showing results of discontinuous epitopes and their 3D structures.*

NO	Number of residues	Structure	NO	Number of residues	Structure
1-	134		5-	53	
2-	127		6-	15	
3-	101		7-	4	
4-	67		8-	3	

3.4. Antigenicity, Allergenicity and Toxicity of Epitopes

Using vaxijen server result showed V35 protein (5XSQ) in Marburg virus is antigenic with default antigenic 0.4184 and no allergy using ALLertop, with no toxicity for epitopes using ToxinPred.

4. Discussion

The new method of prediction and designing vaccines is increase, mostly using software to get little accurate results after laboratory tests. But still few people work on Marburg virus a last research published last year designing a complete vaccine for the seven structural protein virus parts including protein exist around the virus [17]. but in this study focus only on VP35 protein which is also a structural protein lead to generate vaccine to attack a specific part of the virus during its journey inside the body. Another study about VP35 protein was carried last year and also differ from this study research that it contain a drug designed for curing the virus but the same because it use a bioinformatics tools with different software types and follow the same procedure in designing [18]. Another study focus only to design vaccine against Marburg virus T-cell (CD4) and generate cellular immune response [19]. In this research study focus on the two types of immune system response.

5. Conclusion and Recommendation

Since that Marburg virus is lately discovered and still no vaccine or even drug I recommend to do more research on the study at the lab and apply clinical research trial on till reach to the best vaccine to prevent Marburg virus from spreading, and do more research on different location and ethnicity groups especially that are closely living with the bats that carry the virus. Marburg virus is currently look like a dangerous serious one because no treatment nowadays the same as Ebola virus, and because the people suffering from the virus are living in somehow remote area in Africa, for that most scientists not care to find the best cure or vaccine but engaging with people in that area lead to outbreak which if it happen will no way early to stop.

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