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Original Article

EBOLA VACCINE: MULTIPLE PEPTIDE-EPITOPE LOADED VACCINE FORMULATION FROM PROTEOME USING REVERSE VACCINOLOGY APPROACH

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ABSTRACT

Objective: The present study was carried out to identify the peptide epitopes with high immunogenicity in the surface proteins of four pathogenic Ebola virus (*viz.* Bundibugyo virus, Sudan virus, Tai Forest virus and Zaire Ebola virus) using modern reverse vaccinology approach through *in silico* analysis of proteome for use as Ebola vaccine candidates.

Methods: Hexapeptide epitopes based on maximum hydrophilicity were identified in eight surface proteins which were separated from a pool of 160 Ebola virus proteins using a covariant discriminant function and the Mahalanobis D2 statistic. Heptapeptide B cell epitopes were predicted from the surface proteins using the AbDesigner algorithm. Immunogenicity score of each identified epitope was estimated on the basis of hydropathy index and Chou-Fasman conformation.

Results: Four continuous (linear) hexapeptide epitopes namely RRKRRD (position 497-502), DEDDED (489-494), RRTRRE (497-502) and KTGKKG (221-226) with maximum hydrophilicity score were identified from different surface proteins for use as Ebola vaccine components. For use as B cell epitopes eight linear heptapeptide epitopes *viz.* PTSPPQD (418-424) and SHYEPPN (385-391) against Bundibugyo virus, PDYDDCH (309-315) and DYDDCHS (310-316) against Sudan virus, QPKCNPN (508-514) against Tai Forest virus and EYTYPDS (685-691), HLGLDDQ (365-371) and DQEKKIL (370-376) against Zaire Ebola virus with high immunogenicity were identified from different surface proteins of Ebola virus.

Conclusion: Four hexapeptide and eight heptapeptide epitopes can be loaded along with T cell or B cell signal peptides in virus like particle (vlp) or formulated as subunit vaccine by pharmaceutical industry to raise humoral immunity against Ebola virus in African population as well as in other human populations across the globe as therapeutics in the same way the Hepatitis B therapeutic vaccine based on multiple peptide-epitopes was designed nearly a decade ago.

Keywords: Ebola, Vaccine, Peptide epitopes, Immunogenicity, Hydrophilicity, Chou-Fasman conformation.

INTRODUCTION

Ebola virus disease (EVD) broke out as a fatal disease in the recent past in Africa, particularly in Sudan, Congo, Guinea, Sierra Leone, Liberia and Nigeria. The virus is fast spreading to new areas. Within two to three days of contracting the virus, patients show the symptoms of hemorrhagic fever, sore throat and headache, loss of appetite, chest pain, hiccups and shortness of breath, muscle pain, nausea and diarrhea. Functioning of liver and kidneys is decreased and the affected people begin to bleed either internally or externally. The disease has very high mortality rate, often killing between 50 and 90% of the affected people. The first reported case of EVD dates back to 1976 in Sudan where a lady *Mayinga N* died within a few days of contracting the virus. From 1976 through 2013, fewer than 1000 people per year have been infected. But the largest outbreak of Ebola virus has been during July-August 2014 in West Africa. This recent outbreak has created renewed interests among the scientists to take up the research initiative to combat the virus.

Till todate there is no specific treatment for the disease. Efforts are continuing to develop vaccines against Ebola virus, but none exists till todate. Effective vaccines against Ebola virus is the most essential medical intervention to save thousands of African people from frequent Ebola outbreak. Development of proteome-based peptide vaccine could bring an immediate relief in this noble endeavor.

EVD is caused by four pathogenic viruses in the genus Ebola *viz.* Bundibugyo Virus (BDBV), Sudan Virus (SUDV), Tai Forest Virus (TAFV) and Ebola Virus (EBOV, formerly Zaire Ebola Virus). Ebola has the highest pathogenicity and responsible for the largest number of outbreaks in history. The virus is acquired upon contact with the blood or bodily fluids of an infected animal like fruit bat or monkey. Once human infection occurs, the disease may spread between people through medical equipment (syringe, needle), semen, oral exposure etc.

The virions of Ebola virus contain linear, nonsegmented, single-stranded RNA genome of size 19kb. The genome does not possess a 5'-cap and is non-polyadenylated and non-covalently linked to a protein. The Ebola virus genetics is difficult to study due to its virulent nature and affects the researchers who handle them.

Over the past two centuries, quite a good number of vaccines have been used for the treatment and control of infectious diseases such as smallpox [1-4], diphtheria and poliomyelitis [5-6]. Attempts are being made to develop effective vaccines for eliminating several major pathogens of human populations like malaria. Measles, acute respiratory infection and HIV. It is thus imperative to develop novel vaccine formulations to combat the sophisticated pathogens and to promote the right type of protective immunity in human populations.

In general, the traditional vaccines include either whole inactivated or live attenuated microbes. However, these can be unsafe and might produce several side effects, such as inflammation of tissues [7]. In contrast, the vaccines that contain purified or recombinant macromolecules of pathogens such as surface proteins or polysaccharides seem to have minimal side effects [8]. Moreover, the particulate delivery systems, such as liposomes, emulsions, immunostimulatory complexes and virus like particles, augment antigen-specific immune responses [9]. In case of pathogens where no vaccine is yet available or difficult to develop on the basis of the pathogen's polysaccharide (coat protein) pool, it really makes sense to attempt the development of a peptide-based multiple-epitope loaded vaccine as a preventive measure against the pathogen for immediate application using the modern tools of proteomics. Though such proteome derived vaccine may not be so effective in boosting up immunity as the polysaccharide-based vaccine, the former would certainly reduce the risk of contracting the virus and slow down the spread of the virus in a geographical region.

To generate immunological response to a pathogen, the B cells play a key role by making antibodies against the viral antigens, some developing into memory B cells and performing the role of antigen presenting cells. B cells are continuously produced in the bone marrow. B cells are distinct from other lymphocytes, such as T cells and natural killer cells (NK), by virtue of the presence of a protein on the outer surface known as a B cell receptor (BCR). This specialized receptor protein allows a B cell to bind to a specific antigen. When the BCR matches the antigen, the B cell proliferates and secretes a free form of antibodies. The B cell receptors evolve and change throughout the B cell lifespan. The large B cells, often called plasma B cells, secrete large amounts of antibodies which destroy the microbes and make them easier targets for phagocytes. Plasma B cells contain large amounts of rough endoplasmic reticulum (ER), responsible for synthesizing the specific antibody in the cytoplasm. Plasma B cells are generally short lived and undergo apoptosis when the inciting agent *i. e.* the antigen of the pathogen is eliminated.

B cell recognizes the cognate antigen in its native form in the blood or lymph using B cell receptor or membrane bound immunoglobulin. In contrast, the T cells usually recognize their cognate antigen in a processed form as a peptide fragment presented by an antigen-presenting cell's MHC (major histocompatibility complex) molecule to the T cell receptor. A critical feature of the B cells is that they can be activated in a T-cell dependent or independent manner. Detailed discussion on the molecular biology of B cell activation is beyond the scope of this paper. B cells, in fact, constitute a part in the humoral immunity of the adaptive (acquired) immune system. Humoral immunity is the antibody-mediated immune system. B cells also release the cytokines (proteins) which are used in signaling the immune regulatory functions.

In view of the dire need of a vaccine against Ebola, the present investigation was taken up to identify the potential peptide antigens from the extracellular/surface proteins of different pathogenic strains of Ebola and to estimate their immunogenicity using the tools of bioinformatics. The identified peptide antigens with very high immunogenicity could be packaged in a vaccine formulation by the pharmaceutical industry for immediate application to raise protective immunity against Ebola virus in African population to save valuable lives.

Table 1: Hexapeptide epitopes with antigenic propensity in four pathogenic Ebola viruses (Hopp & Woods approach)

Ebola Virus	No. of proteins analyzed	Accession No.	Protein type	Hexapeptide Epitope	Epitope Position	Hydrophilicity score	Antigenic propensity of epitope
Bundibugyo Virus	30	gi|302371218 |YP_3815435.1	Spike glycoprotein	**RRTRRE**	497-502	14.6	5.25
		gi|499104260 |AGL73474.1	Glycoprotein	**RRTREE**	497-502	14.6	5.25
Sudan Virus	66	gi|165940957 |ABY75323.1	Matrix protein	**KTGKKG**	221-226	8.6	5.45
		gi|81933487 |Q5XX06.1	VP 40 protein	**KTGKKG**	221-226	8.6	5.45
Tai Forest Virus	20	gi|1041218 |AAB37093.1	Virion spike glycoprotein	**RRKRRD**	497-502	18.0	5.29
		gi|302315373 |YP_3815426.1	Spike glycoprotein precursor	**RRKRRD**	497-502	18.0	5.29
Zaire Ebola Virus	50	gi|10314000 |NP_066243.1	NP gene product	**DEDDED**	489-494	18.0	5.17
		gi|436409443 |AGB56839.1	Virion spike glycoprotein precursor	**RRTRRE**	497-502	14.6	5.25

MATERIALS AND METHODS

Materials

All the complete protein sequences for each of the four pathogenic Ebola viruses *viz.* Bundibugyo Virus (30), Sudan virus (66), Tai Forest Virus (20) and Zaire Ebola Virus (50) were retrieved from NCBI database (http: //www. ncbi. nlm. nih. gov).

Methods

Using an in-house perl script, the surface or extracellular proteins of each virus were determined out of five probable subcellular locations of prokaryotic proteins on the basis of a covariant discriminant function algorithm and Mahalanobis D^2 statistic [10]. This method requires the determination of the inverse of a 20x20 matrix for each subcellular location. When the determinant of the matrix was found to be zero for the matrix, *Eigen* value-*Eigen* vector system was used to find the inverse matrix for ascertaining the actual subcellular location of each protein.

The surface proteins or extracellular proteins of each virus were separated using bioinformatics tool. The linear antigenic determinant (hexapeptide epitope) of each surface protein was determined based on the highest hydrophilicity score of each hexapeptide from N terminal end of the protein in a sliding window for one amino acid in the protein [11]. Using this technique, one linear hexapeptide epitope for each protein was precisely predicted with exact location in the protein. The antigenic propensity, the hydrophilicity score and the number of aliphatic amino acids (capable of forming interactions with other molecules) of each hexapeptide epitope were estimated for each surface protein.

The B cell epitopes (linear heptapeptides) of each surface protein were determined using the primary structure based algorithm [12] along with its antigenic propensity. In a protein more than one B cell epitope could be found. The antigenic propensity, the hydrophilicity score and the number of aliphatic amino acids for each B cell heptapeptide epitope were calculated. Not all epitopes in a protein induce high humoral immunity *i. e.* produce a high antibody titers against the protein antigens. In order to find out all the continuous B cell epitopes of a protein with high immunogenicity, the AbDesigner algorithm [13] was used to estimate the hydropathy index, and the Chou-Fasman conformational parameter (of beta turn) which were further utilized for estimating the immunogenicity of the 7-mer heptapeptide epitope.

The surface proteins having potential to generate the efficient epitopes for use as vaccines were further analyzed following *in silico* approach for the polar/non-polar amino acids, hydrophilicity, Gravy score, aromaticity, net charge at neutral pH (7.0) and pI (isoelectric point) to elucidate the interrelationships among the surface proteins for each biochemical character.

RESULTS AND DISCUSSIONS

Multi-epitope peptide-based vaccines represent a powerful approach to overcome the immunodominance and thus

simultaneously generate broad immune responses [14-15]. A synthetic-peptide vaccine approach is ideally suited to meet the requirements [16] where different sequences can be easily included in a single dose. Vaccines can be altered to include the emerging variant sequences.

Fig. 1: Antigenic propensity, hydrophilicity and aliphatic amino acids in hexapeptide epitopes of Ebola virus

The emerging vaccine informatics and immunoinformatics-based vaccine design strategies were reviewed [17]. Multi-epitope peptide-loaded virus like particles containing peptide epitopes HBx (52-60), HBx (92-100) and HBx (115-123) derived from the *x* protein of Hepatitis B virus demonstrated significantly higher immunogenicity than any single responsive epitope against the hepatocellular carcinoma [14]. Research work has revealed that the developing countries are at more risk for viral diseases like hepatitis C virus [18]. Taking both the risks and the costs of immunization into consideration, the researchers suggested that a peptide-based vaccine derived from reverse vaccinology approach may be a reasonable prophylactic protection as well as a therapeutic intervention in already infected patients.

They reported two immunogenic HLA-A2-restricted decapeptide core epitopes *i. e.* core 35-44 (YLLPRRGPRL) and core 131-140 (ADLMGYIPLV) to induce peptide-specific cytotoxic T lymphocytes (CTL).

In fact, the development and successful administration of peptide vaccine in human against Hepatitis B virus using genome derived approach more than a decade ago ushered in a new era of vaccinology. These vaccines were prepared primarily on the basis of information generated from the bioinformatic analysis of viral proteomes within two years of whole genome sequencing of the pathogen.

Table 2: Immunogenicity (Ig) analysis of hexapeptide epitopes in four Ebola viruses (Hopp & Woods approach)

Ebola Virus	Accession No.	Hexapeptide Epitope	No. of Aliphatic amino acids	Immunogenicity Analysis of Hexapeptide Epitopes		
				Hydropathy index	Chou-Fasman Conformation	Ig value
Bundibugyo Virus	gi|302371218 |YP_3815435.1	**RRTRRE**	0	8.20	0.92	7.54
	gi|499104260 |AGL73474.1	**RRTREE**	0	8.20	0.92	7.54
Sudan Virus	gi|165940957 |ABY75323.1	**KTGKKG**	5	6.70	1.18	7.91
	gi|81933487 |Q5XX06.1	**KTGKKG**	5	6.70	1.18	7.91
Tai Forest Virus	gi|1041218 |AAB37093.1	**RRKRRD**	1	8.73	1.05	9.17
	gi|302315373 |YP_3815426.1	**RRKRRD**	1	8.73	1.05	9.17
Zaire Ebola Virus	gi|10314000 |NP_066243.1	**DEDDED**	0	8.00	1.22	9.76
	gi|436409443 |AGB56839.1	**RRTRRE**	0	8.20	0.92	7.54

Thus, vaccine formulation, either peptide or DNA vaccine, using bioinformatics tools is a risk-free and rapid process to design the effective vaccines against many fatal diseases, subject to the availability of whole genome or proteome of the particular candidate pathogens against which vaccines need to be developed. From the analysis of 160 proteins in the present study across four pathogenic Ebola viruses (Bundibugyo, Sudan, Tai Forest and Zaire Ebola virus), a total of eight surface proteins, two from each virus, were identified as potential proteins for deriving the epitopes using a covariant discriminant algorithm and Mahalanobis D^2 statistic.

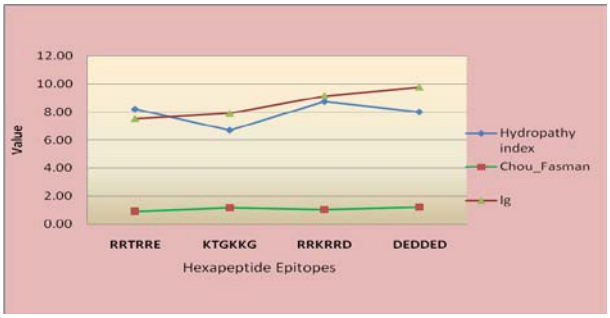

Fig. 2: Immunogenicity of hexapeptide epitopes in Ebola virus

Hexapeptide Epitope Analysis

The antigenic determinants in proteins contain a continuous or linear sequence of 6-10 amino acids. It was observed that hexapeptide antigenic determinants in proteins induce more effective immune response in host against pathogens [11]. One hexapeptide antigenic determinant (epitope) based on maximum hydrophilicity value was detected for each surface protein using the method [11]. Hydrophilicity and antigenic propensity of the hexapeptide epitopes were estimated based on amino acid sequence (Table 1). Two hexapeptide epitopes namely RRKRRD (position 497-502) and DEDDED (489-494) recorded the highest hydrophilicity score of 18.0 with antigenic propensity of 5.29 and 5.17, respectively against Tai Forest virus and Zaire Ebola virus (Fig 1). But the hexapeptide epitope RRTRRE (497-502) recorded maximum hydrophilicity score of 14.6 with antigenic propensity of 5.25 for Bundibugyo and Zaire Ebola virus. Similarly, for Sudan virus the hexapeptide KTGKKG (221-226) was identified with maximum hydrophilicity score of 8.6 and antigenic propensity of 5.45. These hexapeptide sequences are linear 6-mer sequences identified in a 6-mer sliding window moving by one amino acid from N terminal end of each surface protein. These peptides having the highest hydrophilicity score would tend to orient on the surface region of the protein structure and might interact with other molecules to elicit specific antibodies.

For successful use of peptide epitopes as potential vaccine candidates, the epitopes must be highly immunogenic. The immunogenicity of four hexapeptide epitopes was estimated using the immunogenicity score (Ig) derived from hydropathy index and Chou-Fasman conformation for beta turn [13]. Immunogenicity analysis revealed that two hexapeptide epitopes DEDDED (9.76) and RRKRRD (9.17) recorded very high immunogenicity score for Zaire Ebola and Tai Forest virus (Fig 2). But the epitope KTGKKG was estimated to have immunogenicity score of 7.91 in case of Sudan virus whereas the epitope RRTRRE recorded the immunogenicity score of 7.54 for Bundibugyo and Zaire Ebola virus. The peptide epitopes capable of binding to immunoglobulin protein complexes are usually enriched in aliphatic amino acids (*i. e.* A, G, I, K, L, V & M). Amongst four epitopes, the epitopes KTGKKG and RRKRRD had 5 and 1 aliphatic amino acids, respectively whereas the epitopes DEDDED and RRTRRE contained no aliphatic amino acid.

Heptapeptide (7-mer) Epitope Analysis as B cell Epitope

Bioinformatic approach can be applied for the identification of linear heptapeptide B cell epitopes, which are potential vaccine candidates, located in the surface proteins. For each Ebola virus four B cell epitopes were identified with their corresponding position in the complete protein (Table 3).

Hydrophilicity score and antigenic propensity were estimated for each epitope (Fig 3). Hydrophilicity score of epitopes ranged from -5.30 (SSYYHTT, position 210-216) to 8.60 (DQEKKIL, 370-376) whereas the antigenic propensity varied from 6.99 (PSTPPQD, 418-424) to 7.38 (DPSSYYH, 208-214) (Fig 4). The epitopes with positive hydrophilicity coupled with high antigenic propensity could be the potential candidates in subunit vaccine formulation.

Thus the heptapeptide epitopes PSTPPQD and SHYEPPN (385-391) could be used as B cell epitopes against Bundibugyo virus whereas two overlapping heptapeptides PDYDDCH (309-315) and DYDDCHS (310-316) against Sudan virus.

Similarly, the heptapeptide QPKCNPN (508-514) could be a strong B cell epitope against Tai Forest virus but three heptapeptides namely EYTYPDS (685-691), HLGLDDQ (365-371) and DQEKKIL (370-376) are potential vaccine candidates against Zaire Ebola virus. Immunogenicity analysis of the B cell epitopes revealed that immunogenicity score of heptapeptides was in the range 5.28 – 8.58 (Table 4). In fact, all the identified epitopes recorded high immunogenicity score suggesting that these heptapeptides would raise humoral immunity when successfully administered as antigens in carrier particles. The B cell epitopes PTSPPQD, SHYEPPN, PDYDDCH, DYDDCHS and QPKCNPN were estimated to have immunogenicity score more than 8.0 for Bundibugyo, Sudan and Tai Forest virus. The epitopes EYTYPDS for Zaire Ebola virus recorded relatively low immunogenicity score (7.57). The epitopes HLGLDDQ and DQEKKIL had low immunogenicity score 5.86 and 5.28 for Zaire Ebola virus. From the comparison of aliphatic amino composition in B cell epitopes, it was evident that the epitopes DQEKKIL, HLGLDDQ and QPKCNPN contained 4, 3 and 1 aliphatic amino acids, respectively.

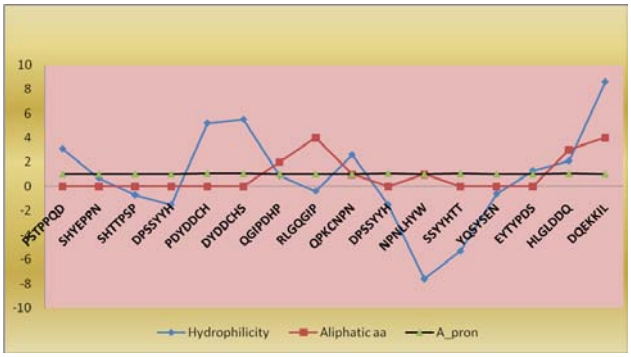

Fig. 3: Antigenic propensity, hydrophilicity and aliphatic amino acids in B cell epitopes of Ebola virus

Table 3: Proteins having potential B cell epitopes for Ebola vaccine development

Ebola Virus	Accession No.	Protein type	B cell Epitope (7mer)	Epitope position in protein	Hydrophilicity score	Epitope's Antigenic propensity
Bundibugyo Virus	gi|302371218 |YP_3815435.1	Spike glycoprotein	PSTPPQD	418-424	3.10	6.99
			SHYEPPN	385-391	0.70	6.99
	gi|499104260 |AGL73474.1	Glycoprotein	SHTTPSP	430-436	-0.70	7.07
			DPSSYYH	208-214	-1.50	7.38
Sudan Virus	gi|165940957 |ABY75323.1	Matrix protein	PDYDDCH	309-315	5.20	7.34
			DYDDCHS	310-316	5.50	7.29
	gi|81933487 |Q5XX06.1	VP 40 protein	QGIPDHP	140-146	0.90	7.14
			RLGQGIP	137-143	-0.40	7.10
Tai Forest Virus	gi|1041218 |AAB37093.1	Virion spike glycoprotein	QPKCNPN	508-514	2.60	7.04
			DPSSYYH	208-214	-1.50	7.38
	gi|302315373 |YP_3815426.1	Spike glycoprotein precursor	NPNLHYW	512-518	-7.60	7.02
			SSYYHTT	210-216	-5.30	7.27
Zaire Ebola Virus	gi|10314000 |NP_066243.1	NP gene product	YQSYSEN	469-475	-0.60	6.99
			EYTYPDS	685-691	1.30	6.99
	gi|436409443 |AGB56839.1	Virion spike glycoprotein precursor	HLGLDDQ	365-371	2.10	7.23
			DQEKKIL	370-376	8.60	6.99

But the heptapeptide epitopes PSTPPQD, SHYEPPN, PDYDDCH, DYDDCHS and EYTYPDS did not contain even a single aliphatic amino acid.

In silico analysis of the chemical properties (hydrophilicity, Gravy score for hydrophobicity, aromaticity, net charge at pH 7.0, pI *i. e.* Isoelectric point and polar/total amino acid ratio) for interrelationships of eight surface proteins revealed that Gravy score, isoelectric point and net charge at pH 7.0 showed a similar trend (Table 5). But hydrophobicity and isoelectric point of the proteins showed almost a reverse relationship (Fig 5).

Peptide vaccines offer several advantages. Synthetic peptide-based vaccines administered subcutaneously in adjuvants are able to induce strong protective immunity against virus infections and malignancies as shown in several murine model systems [19-20].

Therefore, it is a novel strategy to stimulate immune response very purposefully based on a selected epitope or even a mixture of desired epitopes. Using a suitable adjuvant, peptide immunization could be a very straight forward method [18].

Compared to the recombinant or attenuated viruses which are generally very powerful tools for immunization, the peptide-based vaccines do not run the risk of a back-mutation into a dangerous or potential virus as the former does.

Not only are peptides easily produced at low cost, peptide vaccines do not need an uninterrupted cold preservation system.

Apart from the merits, a peptide-based vaccine is often associated with some demerits too. A peptide *per se* is usually not very immunogenic and therefore, the only solution is to choose a suitable adjuvant and carrier in order to protect the peptide from protease degradation and direct it into an immune response pathway.

Furthermore, it may be hard to predict the outcome of a peptide-based vaccine at the initial stage. Depending on the antigen sequence, the dose and the route of application, the peptide immunization strategy could end up with tolerance in the vaccinated individuals, or an ordinary immune response, immunopathology or even autoimmunity.

Table 4: Immunogenicity analysis of B cell epitopes against four Ebola viruses (Kolaskar & Tongaonkar approach)

Ebola Virus	B cell Epitope (7mer)	No. of Aliphatic amino acids*	Immunogenicity Analysis of 7mer Epitopes		
			Hydropathy index	Chou-Fasman Conformation	Ig value
Bundibugyo Virus	**PSTPPQD**	0	6.40	1.34	8.58
	SHYEPPN	0	6.71	1.27	8.52
	SHTTPSP	0	5.84	1.25	7.30
	DPSSYYH	0	6.29	1.30	8.18
Sudan Virus	**PDYDDCH**	0	6.51	1.31	8.53
	DYDDCHS	0	6.40	1.30	8.32
	QGIPDHP	2	5.83	1.21	7.05
	RLGQGIP	4	4.80	1.09	5.23
Tai Forest Virus	**QPKCNPN**	1	6.66	1.33	8.86
	DPSSYYH	0	6.29	1.30	8.18
	NPNLHYW	1	5.96	1.18	7.03
	SSYYHTT	0	5.76	1.14	6.57
Zaire Ebola Virus	**YQSYSEN**	0	6.60	1.20	7.92
	EYTYPDS	0	6.31	1.20	7.57
	HLGLDDQ	3	5.43	1.08	5.86
	DQEKKIL	4	5.93	0.89	5.28

*Aliphatic amino acids considered for analysis: A, G, I, K, L, V & M

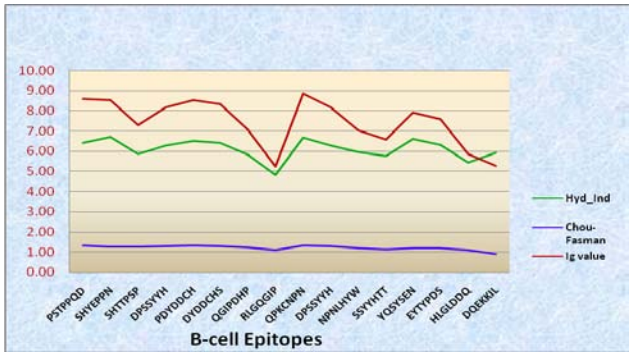

Fig. 4: Hydropathy index, Chou-Fasman conformation and immunogenicity (Ig) of B cell epitopes in Ebola

Table 5: Chemical properties of eight surface proteins in four pathogenic Ebola viruses

Protein	Hydrophilicity (x100)	Total Gravy score (x100)	Aromaticity	Net charge at pH 7	pI	Polar/Total aa
Spike glycoprotein	9.53	-314.70	0.09	-7.21	3.20	0.30
Glycoprotein	9.51	-276.80	0.08	-3.21	3.70	0.30
Matrix protein	4.54	-20.60	0.06	3.60	8.05	0.25
VP 40	4.54	-20.60	0.06	3.60	8.05	0.25
Virion spike glycoprotein	9.52	-222.20	0.09	-4.17	3.70	0.28
Spike glycoprotein precursor	9.52	-216.20	0.09	-3.22	3.70	0.28
NP Gene Protein	10.44	-511.00	0.07	-38.94	3.70	0.37
Virion Spike glycoprotein precursor	9.60	-261.80	0.09	-6.21	3.70	0.29

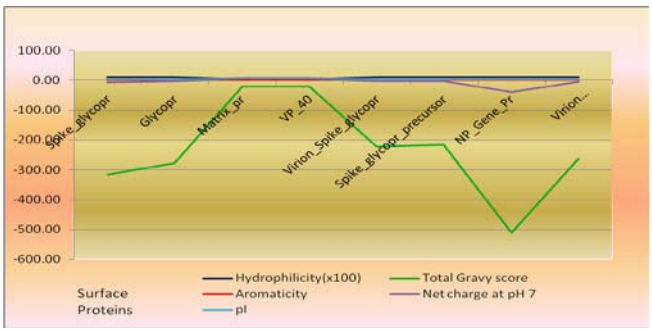

Fig. 5: Chemical properties of eight surface proteins in Ebola virus

Virosomes have been successfully used in vaccine development. Virosomal vaccines against both influenza virus (Inflexal) and hepatitis A virus (Epaxal) already exist in the Swiss market [18]. Virosomes are in fact liposomes and seem to be suitable carriers or vectors for delivering various kinds of antigens such as peptides, proteins and inactivated viruses or even DNA.

Moreover, the complex interactions among components of the vaccine are hard to predict and demand a through pre-clinical test program. Since no effective vaccine is yet available in the market as a prophylactic or therapeutic measure against Ebola virus, it is imperative to develop a multi-epitope peptide-based vaccine formulation against Ebola virus. Further research work may be initiated to understand the reactivity of these epitopes with human proteome.

CONCLUSION

The results of the present study suggest that a multiepitope peptide-based vaccine may be formulated with a mixture of 4 hexapeptide (6-mer) epitopes *i. e.* RRKRRD, DEDDED, RRTRRE and KTGKKG and 8 heptapeptide (7-mer) epitopes *i. e.* PTSPPQD, SHYEPPN, PDYDDCH, DYDDCHS, QPKCNPN, EYTYPDS, HLGLDDQ, and DQEKKIL with T-cell and B-cell targeting (signal) peptides in a virus like particle (vlp) by pharmaceutical industry followed by pre-clinical tests to combat the spread of fatal Ebola virus in human population.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest in this research work.

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