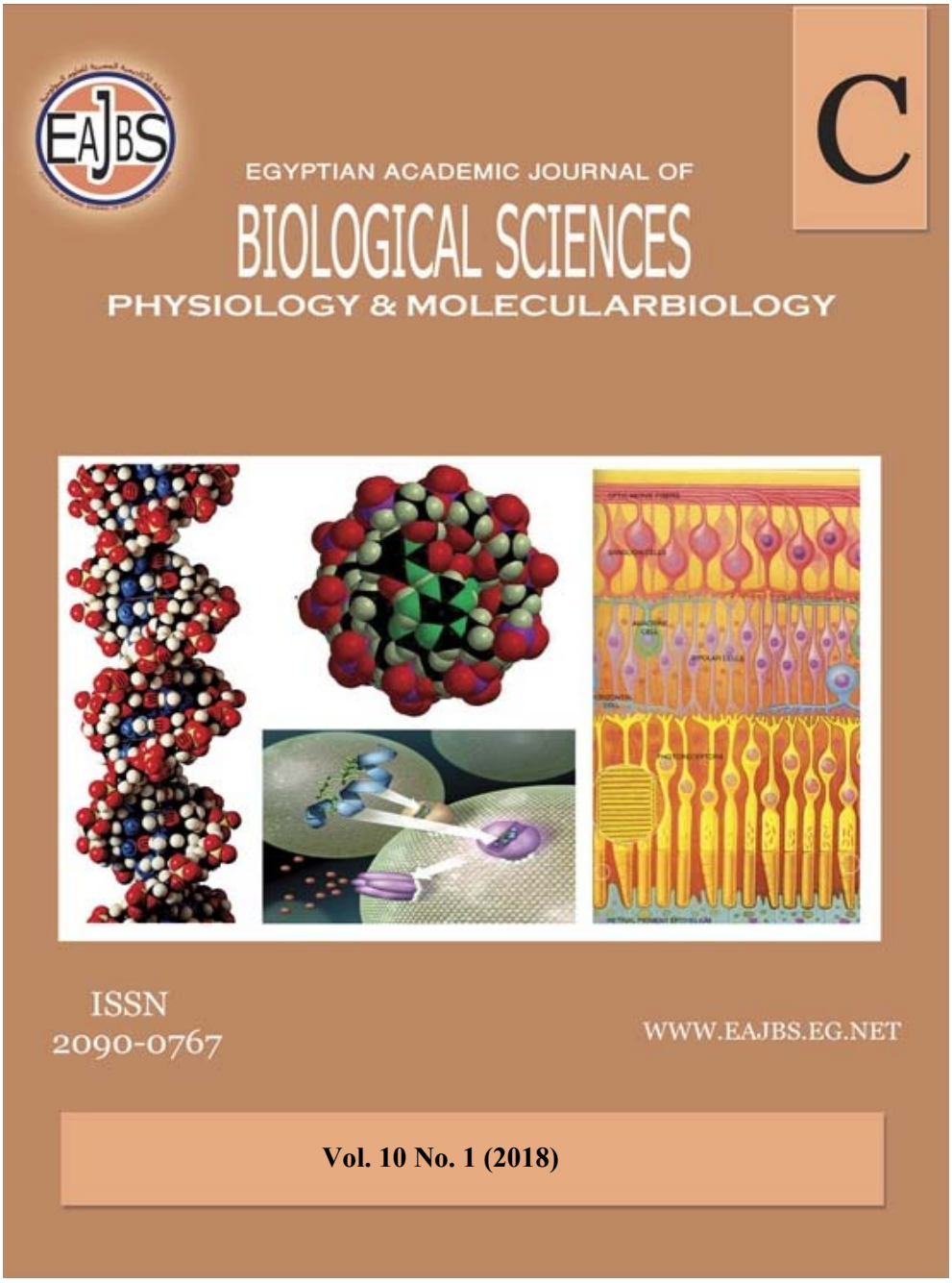

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Prediction of Epitope Based Vaccine Candidates against *Macaca fascicularis* PV Type 2 Virus Using *In-silico* Approaches

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ABSTRACT

Papillomaviruses are the causing agents of benign tumors in their hosts, i.e., mammals and birds, across the world. They have circular double stranded DNA genome. In order to combat the viral infection in *Macaca fascicularis* PV type 2, a computational pipeline was employed in this study for the prediction of viral protein targeting peptides for vaccine discovery. Epitope prediction enabled the identification of multi-peptides suitable for vaccine development. Further in-depth analysis for immunogenicity and toxicity prediction scrutinized the optimal candidate for target based designing of vaccines. Immunogenic and physicochemical properties of proteins E1, E2, E4, E6, E7, L1, and L2 of *Macaca fascicularis* PV type 2 revealed their instability index, molecular weight, and antigenic potential. The predicted epitopes may lead to promising targets for broad spectrum vaccine designing against the viral strain of *Macaca fascicularis* PV type 2.

INTRODUCTION

Papillomaviruses are double stranded DNA viruses identified in healthy skin and melanoma skin from immunocompetent patients (Forslund *et al.*, 2007). They are associated with mucosal, oral (Parkin and Bray, 2006), epithelial, and cervical cancers (zur Hausen, 2002). They are mainly classified as alphapapillomavirus, betapapillomavirus, chipapapillomavirus, deltapapillomavirus, gammapapillomavirus, etapapillomavirus, and epsilonpapillomavirus. Betapapillomaviruses are double stranded non-enveloped circular DNA viruses with icosahedral geometries (Bernard *et al.*, 2010; Chan *et al.*, 1997; Chen *et al.*, 2009; de Villiers *et al.*, 2004).

Macaca fascicularis PV type 2 (MfPV2) belongs to betapapilloma group of the family, etiological agent of both benign, and malignant skin lesions mainly in primates similar to that is seen in human population (Chen *et al.*, 2009). These include rhesus (*Macaca mulatta*) (Chan *et al.*, 1997), cynomolgus macaques (*Macaca fascicularis*) (Antonsson and Hansson, 2002), and pygmy chimpanzees (*Pan paniscus*) (Van Ranst *et al.*, 1991). Sequence similarity search and phylogenetic analysis reveals *Macaca fascicularis* PV type 2 genome is closely related to human papilloma virus type 124 with 92% similarity.

In order to combat the infection caused by *Macaca fascicularis* PV type 2, there is an urgent need to develop effective therapeutic vaccine providing protection at inter- and intra-species level. Overcoming the limitation caused by time consuming, labor intensive, and expensive traditional methods of generating monoclonal antibodies, *in silico* epitope identification is considered as a potential route of the development of broad spectrum vaccine. Invading pathogenesis by identifying B-cell epitopes initiating humoral immune response by antigen-antibody interaction (Getzoff *et al.*, 1988; Somvanshi and Seth, 2009) along with antigens binding to HLA class I (CD8+ T-cells) and HLA class II (CD4+ T-cells) alleles with specificity and sensitivity was used earlier against several viruses (Singh *et al.*, 2009; Somvanshi *et al.*, 2008a; Somvanshi *et al.*, 2008b). Additionally, population coverage analysis was included in the study to identify the epitope(s) restricting the limitation of extreme polymorphism among maximal population setting. The consequential epitopes of the present study would be a germane initiator for potential vaccine development against *Macaca fascicularis* PV type 2.

MATERIALS AND METHODS

Data-set Collection:

A complete set of bioinformatics tools and softwares were used in a sequential manner for the complete analysis, epitope prediction, and characterization of *Macaca fascicularis* papilloma virus type 2 genome. The structural protein sequences of *Macaca fascicularis* papilloma virus type 2 were retrieved from NCBI Genome database in GenBank format (https://www.ncbi.nlm.nih.gov/genome/). The complete genomic sequence was subjected to Open Reading Frame (ORF) finder (https://www.ncbi.nlm.nih.gov/orffinder) for the identification of open reading frames.

Physicochemical Characterization:

ProtParam (Gasteiger *et al.,* 2005), an online protein analysis tool on EXPASY server was used for the appraisal of various physicochemical properties including molecular weight, amino acid composition, extinction coefficient (Gill and Hippel, 1989), theoretical pI, and grand average of hydropathicity (Kyte and Doolittle, 1982), aliphatic index (Ikai, 1980), and instability index (Guruprasad *et al.,* 1990).

Prediction of Immunogenicity and Toxicity Assessment:

The immunogenic potential of the protein sequences was predicted using an alignment independent antigen prediction immunoinformatics tool, VaxiJen server V2.0 (http://www.ddg-pharmfac.net/vaxijen/Vaxi-Jen/VaxiJen.html) based on physicochemical properties of proteins and toxic potential using ToxinPred (Gupta *et al.*, 2013).

Epitope Prediction:

Possible epitopes (B-cell and T-cell for MHC Class I & II) in protein primary sequences were screened out using ABCpred server (www.imtech.res.in/abcpred) and Immune Epitope Database tool (www.iedb.org). Both tools involve combinatorial machine learning algorithmic approach for epitope prediction. Peptide property calculator (https://www.genscript.com), a freely available tool to determine the best solvent for a peptide was used for peptide property calculation.

Population Coverage Analysis:

Afterwards, population coverage analysis was done by using Immune Epitope Database and Analysis Resource (IEDB) Population Coverage tool available at http://tools.immuneepitope.org/tools/population/iedb_input for the identification of all the possible binding alleles (MHC Class I and MHC Class II)

with respect to the identified T-cell epitopes (Bui *et al.*, 2006).

RESULTS AND DISCUSSION

Macaca fascicularis PV type 2 (MfPV2) genome, isolated from exophytic skin of hand and feet of cynomolgus monkey (*M. fascicularis*) is a double stranded DNA virus of size 7632 base pairs and includes seven proteins (E1, E2, E4, E6, E7, L1, and L2). The complete genomic sequence was retrieved from NCBI Genome database (NC_015691) in GenBank format. In the present study, sequence based analysis along with physicochemical characterization, epitope prediction and population coverage analysis was accomplished on the protein sequences of *Macaca fascicularis* PV type 2. A highly proficient computational pipeline involving bioinformatics tools was developed to retrieve a vast amount of data to identify potential vaccine candidates. In order to predict the biological activity of the proteins, physicochemical characterization was performed. The physicochemical properties of the identified proteins (Somvanshi and Seth, 2009) were computed using ProtParam server (Table 1).

Table 1: Immunogenic and physicochemical properties of proteins of *Macaca fascicularis* PV type 2.

Protein	Molecular Weight	Amino acid Composition	Extinction Coefficient	Theoretical pI	Aliphatic Index	Instability Index	GRAVY	VaxiJen Score
E1	70633.61	614	104695	5.17	76.07	52.94	-0.478	0.4486 (probable antigen)
E2	50780.02	454	68090	10.2	61.87	59.19	-0.761	0.6068 (probable antigen)
E4	22885.45	204	22460	4.61	60.64	54.26	-1.218	0.5820 (probable antigen)
E6	15710.16	139	30075	7.50	90.58	45.26	-0.55	0.7369 (probable antigen)
E7	12358.10	108	1740	4.97	92.87	52.2	-0.409	0.4566 (probable antigen)
L1	57911.27	513	71780	6.18	78.28	52.15	-0.448	0.4568 (probable antigen)
L2	56476.09	526	23965	4.90	82.09	42.97	-0.264	0.4903 (probable antigen)

The instability index is an estimate of the stability of a protein in a test tube, consequently seven proteins were found stable in nature ranged between (42.97–59.19). The sequential addition of hydropathy values of each amino acid residue divided by the number of amino acids is the indicator of protein hydrophobicity (GRAVY). It is calculated as a sum of *hydropathy* values of all the amino acids residues divided by the number of residues in the sequence. Increasing positive score shares directly proportional relationship with hydrophobicity. Theoretical isoelectric point deciphers pH dependent characteristics of a protein in a suitable medium ranging between 4.61–10.2. Immunogenicity prediction declares the potential of the proteins to acts as probable antigen.

Information about the epitopic regions or antigenic determining factor is necessary for designing active inhibitors in contrast to active viral proteins. T-cell and B-cell antibodies recognize a specific part of antigen to bind with specificity, termed as epitope or antigenic determinant. Antibodies recognize specific regions (antigenic determinants or B-cell epitopes) and bind to the antigens with the specificity. Understanding the antigen-antibody interaction pattern determine the viral pathogenesis. B-cell epitopes were determined for seven proteins of *Macaca fascicularis PV* type 2 (Table 2).

Table 2: Predicted B-cell epitopes in *Macaca fascicularis* PV type 2.

Proteins	Predicted B-cell epitopes
E6	MAGRPLSATALAEE GI KDLQV STLSGA GVAYAACSPCCAATAAYEVQ SVYGREIERITNTP DICAR
E1	MGDSRGTDDVKPGCSDW AECSDIEND KLFEDDTDSNISGLIDDGDVIQGN QLSP DSGVDLTQNEVEDIPEEEVEVPTDSVDNVPAPAVQEPAVQGVRGQGSL RQYKSNKTC SAKSRD ISEPPRLR HHTGECPQF AYDNDYVEEADIA KLADEDANARAWLSSNSQAK WGPPDTGKST KFPFKDDGTPQFNLTDQSW
E2	SGKNT QKAKD KSPYGGESW ETFRSPPQQCFKKGGQEVEVRFDGDPE HDSWQKVQGQVD TDAKRYG VTSSTPEGQGSPAPADSNTAEGSQHPTEVVSVDSATTSAPAATSATAKQRARRYG RKASSPGGGERGYSPLKENQRER RPHKRGRGERGGRSRSRSRATSRSRSRSRSRTRTRARPQSATTTHPCTRSRSRSR GGVLPSQ WVGADNNDRI
E4	PPPLPPQPPLPPLNSEPEGTGEKPPVQEEENEDTRPSKKTKENGDHTSGDGEKEGG DPDPGPVPHPDPDPDPDPEPEPEPD PNPQPQPIPAPDPDLDP
E7	MIGKEPTI EVLEEQVEEEPEKNPF GGGCG CRETTREETRQRDLN
L1	GKVYLPPSTPVARVQSTDEYI NSTGDKVEVPKVSGN IYDPDKE EIGRGQPLGVGTTGHP DTENPRQYPPQGTKDDRQDVSFDPK CIGEHWDRAKACAGVDQTGLCP TTIQDGDM DIIPDGTVNQDHKYYLPGDSGGPRSTL AQGHNN STEAAGGDSYDATK TRCPDQEPPKEPEDPYAQ
L2	AAGTCPQD ISTGRGSGGATGYTPLGGPGVRVG VIRPGVAPEVVGPS GTIDPSAPSV TLTEGGPDLLPG EISPVPDVASV VAPEPAPPTRTRISQQQYHN VTPSRGETSHG IGGQTVGGAATRGPT IAEPTPPRQTSTPVQ HSGDASVVQGSA QDVPGVSSDSPEYSDAY FSTTRNASYYTQ TSDSSGD VSYPEQRQF

Paratope is a part of an antibody, assists in recognizing the antigenic determinant. Based on the foreignness characteristics, epitopes are typically non-self-protein sequences resulting from the host that can be recognized are also epitopes. Two classes of proteins antigens are known based on amino acid sequence composition (a) conformational epitopes and (b) linear epitopes (Huang and Hond, 2006). Discontinuous segments of the antigen amino acid sequence create conformational epitopes. A single epitope 'PPPLPPQPPLPPLNSEPEGTGEKPPVQEEENEDTRPSKKTKENGDHTSGDGEKEGGDPDPGPVPDPPEPEPDPDPDPEDPNPQPQPIPAPDPDLDP' generated by E4 showed maximum immunogenic potential. Further, considerable potential was generated by L2 protein 'ISTGRGSGG ATGYTPL GGPGVRVG', 'VIRPGVAPEVVGPS', and 'VAPEPAPPTRTRISQQQYHN'. Only a single epitope 'PPPLPPQPPLPPLNSEPEGTGEKPPVQEEENEDTRPSKKTKENGDHTSGDGEKEGGDPDPGPVPHPDPDPDPDPEPEPEPDPNPQPQPIPAPDPDLDP' was generated by envelope protein E4. The desired immune responses are generated by antigenic determinants. Therefore, T cell epitopes identification was performed by Immune Epitope Database tool. The majority of T-cell epitopes depicting highest binding affinity with MHC Class I was shown by E4 and E2 peptides. T-cell epitope 'FFGGLGIST' of protein L2 shows maximum 58 number of MHC Class I. Additionally, 'VDNVPAPAV' of E1 peptide shows binding number with MHC Class I of 24. T-cell epitopes having MHC alleles for the proteins of *Macaca fascicularis* PV type 2 were identified (Table 3).

Table 3: Predicted T-cell epitopes (MHC Class I and MHC Class II) in *Macaca fascicularis* PV type 2.

Proteins	T-cell epitopes	Number of MHC Class I binding alleles	T-cell epitopes	Number of MHC Class I binding alleles
E1	YIWLSSVGA	22	FRRLWSQLD	21
	VDNVPAPAV	34	ITSNYNIMV	09
	FVRECAQMV	11		
	YAKLADEDA	19		
E2	YQPVPNLAI	02	VGADNNDRI	30
	YESGKNTIA	11	FYFDGPVKV	15
	WVGADNNDR	28	ITEQKAKDA	18
			YRGLYTRMS	07
			YGRKASSPG	16
E4	LSLLLSPVQ	22	VQEEENEDT	19
	LSIIRTLSL	31	IIRTLSLLL	27
			VKLDFGRLS	31
			LPPLNSEPE	14
E6	YAACSPCCA	31	WKEGVAYAA	11
	VHYEFSVYG	02	LDLLEKNDI	26
	IERITNTPL	15	WKGNCRHCR	28
			YGREIERIT	19
E7	FKVLVSCGG	11	IGKEPTIRD	16
	FVTATAYGI	09	LCPECRETT	18
	LRLFVTATA	23	LSILCPECR	21
L1	WDRAKACAG	25	YRYLNSLAT	16
			FGAINNITL	26
			MVDNTRNTN	30
L2	YTPLGGPGV	16	FSETPAGYI	22
	YTFEIAEPT	32	LQDVPGVSS	17
	YRDLSSIDT	08	VASVDAPVV	05
	FFGGLGIST	58		
	YRGCKAAGT	15		
	VAPEPAPPT	24		
	IIPLDSLGT	19		

Their immunogenic potential correlates with other viruses and laid the foundation of synthetic biology by developing synthetic peptides for vaccine development. In addition, toxicity prediction enabled behavioral study of peptides under different environmental conditions.

The population coverage analysis depicted potential application of the probable vaccine at a global level by providing maximal coverage ranging from 36.09–99.09% (Table 4 & Figure 1). The population setting at global level was analyzed showing maximum percentage at South East Asia and Central Africa.

Table 4: Population coverage analysis (%) of MHC Class-I and MHC Class-II.

S.No.	MHC Class I				MHC Class II		
1	**Population**	**Coverage**	**Average hit**	**PC90**	**Coverage**	**Average hit**	**PC90**
2	East Asia	88.02%	2.08	1.62	98.99%	2.61	0.78
3	North East Asia	81.88%	1.98	0.43	94.67%	2.00	1.08
4	South Asia	93.05%	1.07	1.09	98.05%	1.47	1.99
5	Southeast Asia	99.02%	1.88	1.11	96.84%	1.37	1.65
6	Europe	83.55%	2.61	0.65	98.01%	1.47	0.43
7	Southwest Asia	87.38%	2.00	0.76	91.16%	1.99	0.78
8	East Africa	76.09%	1.97	0.88	97.44%	1.68	0.69
9	West Africa	64.22%	1.37	1.76	98.17%	1.62	1.09
10	North Africa	36.09%	1.47	0.44	95.77%	2.08	1.11
11	South Africa	78.99%	1.99	0.79	94.87%	1.65	0.54
12	Central Africa	94.77%	2.76	1.23	88.18%	1.47	0.34
13	North America	13.95%	2.41	0.35	97.83%	2.65	0.48
14	Central America	86.75%	1.68	0.54	95.17%	1.09	0.58
15	South America	15.59%	1.62	0.79	99.08%	1.48	0.91
16	West Indies	79.59%	2.08	1.35	97.54%	1.43	0.58
17	Oceania	89.45%	1.65	1.06	89.17%	1.23	0.66
Average		**73.02**	**1.91**	**0.92**	**95.68**	**1.705**	**0.855**

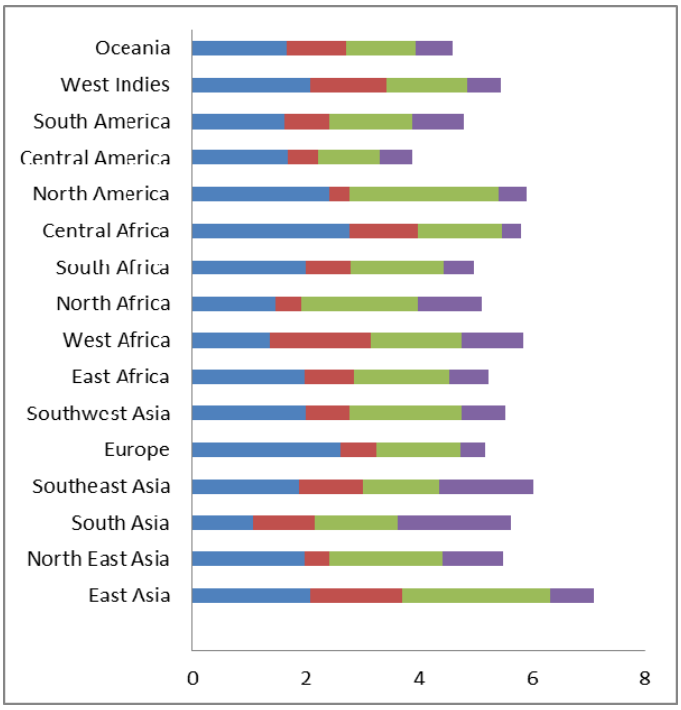

Fig. 1: Representing average hit and PC90 analysis for MHC Class I and MHC Class II, respectively (**blue** = average hit for MHC Class I, **red** = PC90 for MHC Class I, **green** = average hit for MHC Class II, and **purple** = PC90 for MHC Class II).

Conclusion:

The present study dealt with potential vaccine development against *Macaca fascicularis* PV type 2 infection by employing *in silico* strategies. T-cell and B- cell epitopes were successfully identified as they are the driving factor for the primary and secondary humoral immunity. The potential epitopes against MHC Class I and MHC class II were screened and considered as the most probable candidates for vaccine development against *Macaca fascicularis* PV type 2.

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Conflict of Interests:

The author reports no conflict of interests.

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REFERENCES

Antonsson A, Hansson BG (2002) Healthy skin of many animal species harbors papillomaviruses which are closely related to their human counterparts. J Virol 76(24):12537–12542.

Bernard HU, Burk RD, Chen Z, van Doorslaer K, Hausen HZ, de Villiers EM (2010) Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. Virology 401:70–79.

Bui HH, Sidney J, Dinh K, Southwood S, Newman MJ, Sette A (2006) Predicting population coverage of T-cell epitope-based diagnostics and vaccines. BMC Bioinformatics 7:153.

Chan SY, Bernard HU, Ratterree M, Birkebak TA, Faras AJ, Ostrow RS (1997) Genomic diversity and evolution of papillomaviruses in rhesus monkeys. J Virol 71(7):4938–4943.

Chan SY, Ostrow RS, Faras AJ, Bernard HU (1997) Genital papillomaviruses (PVs) and epidermodysplasia verruciformis PVs occur in the same monkey species: implications for PV evolution. Virology 228:213–217.

Chen Z, van Doorslaer K, DeSalle R, Wood CE, Kaplan JR, Wagner JD, Burk RD (2009) Genomic diversity and interspecies host infection of Macaca fascicularis papillomaviruses (MfPVs) within the alpha papillomavirus α12 species. Virology 393:304-310.

de Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H (2004) Classification of papillomaviruses. Virology 324:17-27.

Forslund O (2007) Genetic diversity of cutaneous human papillomaviruses. J Gen Virol 88:2662-2669.

Gasteiger E, Hoogland C, Gattiker A, Duvaud S, Wilkins MR, Appel RD, Bairoch A (2005) Protein Identification and Analysis Tools on the ExPASy Server; (In) John M. Walker (ed): The Proteomics Protocols Handbook, Humana Press: 571-607.

Getzoff ED, Tainer JA, Lerner RA, Geysen HM (1988) The chemistry and mechanism of antibody binding to protein antigens. Advances in Immunology 43:1–98.

Gill SC, von Hippel PH (1989) Calculation of protein extinction coefficients from amino acid sequence data. Anal Biochem 182:319–326.

Gupta S, Kapoor P, Chaudhary K, Gautam A, Kumar R (2013) In silico approach for predicting toxicity of peptides and proteins. PLoS ONE 8:e73957.

Guruprasad K, Reddy BV, Pandit MW (1990) Correlation between stability of a protein and its dipeptide composition: a novel approach for predicting in vivo stability of a protein from its primary sequence. Protein Eng 4:155–161.

Huang J, Hond W. (2006) CED: a conformational epitope database. BMC Immunol 7:7 https://doi.org/10.1186/1471-2172-7-7.

Ikai A (1980) Thermostability and aliphatic index of globular proteins. J Biochem 88:1895–1898.

Kyte J, Doolittle RF (1982) A simple method for displaying the hydropathic character of a protein. J Mol Biol 157:105–132.

Parkin DM, Bray F (2006) Chapter 2: the burden of HPV-related cancers. Vaccine 24(Suppl 3):S11–S25.

Singh V, Indramani, Chaudhary DK, Somvanshi P (2009) HLA class I and II binding promiscuity of the T cell epitopes in putative proteins of Hepatitis B virus. Journal of Computer Science & Systems Biology 2:069-073.

Somvanshi P, Seth PK (2009) Analysis of antigenic diversity of T cell epitopes in the prediction of distinct variants of dengue virus by in silico methods. Indian Journal of Biotechnology 8(2):193-198.

Somvanshi P, Singh V, Seth PK (2008) In silico prediction of epitopes in virulence proteins of Mycobacterium tuberculosis H37Rv for diagnostic and subunit vaccine design. J Proteomics & Bioinformatics 1:143-153.

Somvanshi P, Singh V, Seth PK (2008) Prediction of epitopes in hemagglutinin and neuraminidase proteins of Influenza A virus H5N1 strain: A clue for diagnostic and vaccine development. OMICS: J Integrative Biology 12:61-69.

Somvanshi P, Seth PK (2009) Comparative proteome analysis of distinct variants of dengue virus using in silico methods. Internet Journal of Genomics and Proteomics 4(1).

Van Ranst M, Fuse A, Sobis H, De Meurichy W, Syrjanen SM, Billiau A, Opdenakker G (1991) A papillomavirus related to HPV type 13 in oral focal epithelial hyperplasia in the pygmy chimpanzee. J Oral Pathol Med 20(7):325–331.

zur Hausen H (2002) Papillomaviruses and cancer: from basic studies to clinical application. Nat Rev Cancer 2:342–350.

ARABIC SUMMARY

تحديد مستقبلات الاجسام المضادة للقاح المرشح ضد فيروس ماكاكا فاسيكيولاريس من النوع الثاني باستخدام أسلوب المحاكاة بالحاسوب

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يعد فيروس الورم الحليمي عاملاً مسبباً لظهور الاورام الحميدة في الحيوانات العائلة لهذا الفيروس مثل الثديات والطيور في جميع أنحاء العالم .

الحمض النووي لهذا الفيروس يتألف من خيطين ومن أجل التصدي للإصابة الفيروسية من الفيروس ماكاكا فاسيكيولاريس من النوع الثاني فقد تم استخدام نموذج محاكاة حسابي لتحديد مستقبلات الاجسام المضادة من الببتيدات وذلك لاكتشاف اللقاح المضاد وتحديد مستقبلات الاجسام المضادة لذلك الفيروس من الببتيدات والتي سوف تساعد على تطوير اللقاح المناسب . تم إجراء دراسة أكثر عمقاً لتقدير السمية وكذلك القدرة على تنشيط جهاز المناعة بشكل دقيق لتحديد البروتين الأمثل والذي سوف يستهدفه اللقاح الذي سوف يتم تصميمه .

أوضحت الدراسة المناعية والكيموفيزيائية لصفات البروتينات E1 ,E2.E4.E6.E7.L1.L2 لفيروس ماكاكا فاسيكيولاريس من النوع الثاني مؤشراً لعدم الثبات ، الوزن الجزئي وكذلك قدرتها المناعية .

يبدو أن مستقبلات الاجسام المضادة التي تم تحديدها قد تكون أهداف مناسبة ذات مدى واسع للقاح المصمم ضد فيروس ماكاكا فاسيكيولاريس من النوع الثاني.