A STUDY ON HEAMPHILUS INFLUANZAE TYPE B DISEASE CAUSING ANTIGENS: AN APPROACH OF EPITOPE PREDICTION, ANTIGENICITY AND IMMUNOGENICITY PREDICTION

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REVIEW ARTICLE

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ABSTRACT

Vaccines work by mimicking disease agents and stimulating the immune system which in turn builds a defence mechanism against the disease causing agents. Some of the vaccines contain a part of the disease causing agents which are either weakened or dead. Apart from using vaccines only for viral infections, utilizing the same against Cancers both as therapeutic and preventative has captured huge interest. The use of Cancer vaccines in cancer therapies is called immunotherapy which is done either by specific cancer vaccine or universal cancer vaccine which contain tumour antigens that stimulate the immune system which in turn initiate various mechanisms that terminate tumour cells and prevents recurrence of these tumours. Hemophilus influanzae is a disease causing virus & here we have done a brief study about the different antigens of the b strain of Haemophilus influenzae and compare them using various bioinformatics tools to get the most effective antigen of the above mentioned strain.

Keywords: Heamphilus Influanzae, Antigenicity, Immunogenicity, epitope prediction.

INTRODUCTION

Haemophilus influenzae is an important human-restricted Gram-negative bacterial pathogen, which can cause severe invasive disease, such as meningitis, sepsis, and bacteremic pneumonia in susceptible individuals. Some strains of H. influenzae have a polysaccharide capsule representing the major virulence factor and antigen of this bacterial species. On the basis of the antigenic properties, six serotypes of encapsulated H. influenzae are distinguished (a, b, c, d, e, and f), and there are also non-encapsulated or nontypeable influenzae H. (NTHi). Encapsulated strains exhibit a higher ability to cause invasive disease because the capsule prevents complement-mediated bacteriolysis in the absence of opsonizing antibody. (1,2)Normal individuals can carry H. influenzae in their naso- and oropharynx, and the carriage is considered as the major factor inducing the development of natural immunity against the pathogen, along with exposure to some crossreactive environmental antigens. The invasive disease mostly affects young children (below 2 years of age), as well as the elderly and immunocompromised individuals. One particular serological variant, H. influenzae serotype b (Hib), was the major cause of bacterial meningitis in young children worldwide before the conjugate Hib vaccine became available in the late 1980s. Pediatric vaccination against Hib has resulted in a dramatic decrease in the incidence rates of invasive Hib disease in all countries where the vaccine has been included in the national immunization programs. However, Hib vaccination does not confer protection against other serotypes of H. influenzae. .Until recently, the significance of other serotypes of H. influenzae in the etiology of invasive bacterial infections has been largely overshadowed by Hib. (3, 4) However, it is obvious that other serological types of H. influenzae besides Hib cause significant

morbidity and mortality; moreover, their prevalence appears to be increasing in the Hib vaccine era. During the last decade, an increase in the prevalence of infections caused by NTHi has been reported worldwide, suggesting strain replacement following elimination of Hib from populations with high Hib vaccine coverage. as a new ecological niche became available for colonization with non-Hib strains of H. influenzae. Although an alarming trend towards an increase in the incidence of severe disease caused by NTHi has been now recognized in many countries, less attention is paid to H. influenzae serotype a (Hia), which appears to be present in certain geographic regions and among specific populations only. As most of cases of Hia disease are sporadic, the published reports are not always consistent in their findings. While invasive Hia disease has suffered from inadequate surveillance worldwide, Hia is now recognized as an important pathogen causing serious disease comparable to Hib in severity and case-mortality rates. For example, the case-fatality rate of invasive Hia disease among pediatric cases reported by the Canadian Immunization Monitoring Program ACTive (IMPACT) centers in 1996-2001 reached 16%. Remarkably, the highest incidence rates of invasive Hia disease have been found in some indigenous populations, such as North American Indians and Inuit of Alaska and Northern Canada, reaching the order of magnitude of the incidence rates of Hib in the pre-Hib vaccine era. The reasons for an increased susceptibility to Hia infection specific populations groups among are unknown. The goal of this paper is to summarize the current knowledge on Hia global epidemiology and to discuss potential prevention of this infection using specific immunization. (5, 6)

Clinical features

Clinical categories of invasive disease caused by Hib include meningitis, epiglottitis and a range of other infections such as septic arthritis, cellulitis and pneumonia. Hib is rarely isolated from the blood without a focal infection such as the above being evident or developing subsequently. The classical clinical signs of meningitis - neck stiffness and photophobia - are often not detected in infants, who present with drowsiness, poor feeding and high fever. **Epiglottitis** (inflammation of the epiglottis) presents with respiratory obstruction, associated with soft stridor and often drooling in a pale, febrile, anxious child who remains upright to maximize his or her airway. Meningitis and epiglottitis are almost invariably fatal without appropriate treatment. The case-fatality rate for Hib meningitis in developed countries is at least 3% even with treatment and 15 to 30% of survivors have permanent neurological sequelae. There are no specific clinical features of any of the focal infections to Hib that enable them to due be differentiated from those due to other organisms. However, before the introduction of Hib vaccines, epiglottitis was due to Hib in over 95% of cases. (7-9) Non-typeable influenzae Haemophilus strains may occasionally cause invasive disease, but are a common cause of otitis media in children and bronchitis in adults. Hib vaccines are not effective in preventing NTHi infections.

Structure and growth factor

Haemophilus influenzae is a gram-negative coccobacillus. It is generally aerobic but can grow as a facultative anaerobe. In vitro growth requires accessory growth factors; including "X" factor (hemin) and "V" factor (nicotinamide adenine dinucleotide [NAD]). Chocolate agar media are used for isolation. H. influenzae will generally not grow on blood agar, which lacks NAD. The outermost structure of H. influenzae is composed of polyribosyl-ribitol-phosphate (PRP), a polysaccharide that is responsible for virulence and immunity. Six antigenically and biochemical distinct capsular polysaccharide serotypes have been described; these are designated types a through f. In the prevaccine era, type b organisms accounted for 95% of all strains that caused invasive disease.

Disease

Invasive disease caused by H. influenzae type b can affect many organ systems. The most common types of invasive disease are meningitis, epiglottitis, pneumonia, arthritis, and cellulitis. Meningitis is infection of the membranes covering the brain and is the most common clinical manifestation of invasive Hib disease, accounting for 50%-65% of cases in the prevaccine era. Hallmarks of Hib meningitis are fever, decreased mental status, and stiff neck (these symptoms also occur with meningitis caused by other bacteria). Hearing impairment or other neurologic sequelae occur in 15%-30% of survivors. The case-fatality rate is 2% - 5%, despite appropriate antimicrobial therapy. (10)

Epiglottitis is an infection and swelling of the epiglottis, the tissue in the throat that covers and protects the larynx during swallowing. Epiglottitis may cause life-threatening airway obstruction.

Septic arthritis (joint infection), cellulitis (rapidly progressing skin infection which usually involves face, head, or neck), and pneumonia (which can be mild focal or severe Empyema) are common manifestations of invasive disease.

Cellulitis is a bacterial infection involving the skin. It specifically affects the dermis and subcutaneous fat. Signs and symptoms include an area of redness which increases in size over a couple of days. The borders of the area of redness are generally not sharp and the skin may be swollen. While the redness often turns white when pressure is applied this is not always the case. The area of infection is usually painful. Lymphatic vessels may occasionally be involved and the person may have a fever and feel tired

METHODS AND MATERIAL USED

Epitope prediction

An epitope, also known as antigenic determinant, is the part of an antigen that is recognized by the immune system, specifically by antibodies, B cells, or T cells. The part of an antibody that recognizes the epitope is called a paratope. Although epitopes are usually non-self proteins. sequences derived from the host that can be recognized are also epitopes. Prediction of antigenic epitopes on protein surface is important for vaccines design or we can say that it is a prediction of protein surface regions that are preferentially recognized by antibodies (antigenic epitopes) can help in the design of vaccines components and immune diagnostic reagents. So from this we can predict the surface of an antigen or a foreign material and through this we can design a particular drug for haemophilus influenza.

Antigenicity

The ability to cause the production of antibodies. The degree of antigenicity of a substance depends on the kind and amount of that substance and on the degree to which the host is sensitive to it and able to produce antibodies also called immunogenicity. Antigenicity is the capability of a chemical structure an antigen to bind specifically with a group of certain products that that have adaptive immunity. Antigenicity was more commonly used in the past to refer to what is now known as immunogenicity.

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Table 1. Model actimates of excess and deaths from Ulb diseases with and without Ulb usering delayers in the 2004 Parson

Hib, Haemophikus influenzae type b

Immunogenicity:

The property to being able to induce a specific immune response or a degree to which a substance is able to stimulate immune response is called immunogenicity. Or it is the ability of a particular substance such as an antigen or epitope to provoke an immune response in the body of a human or animals. Immunogenicity differentiates in two categories wanted and unwanted. Wanted

anti-drug-antibodies.

immune response against an antigen which is

undesired. Unwanted immunogenicity is a

strongly linked with therapeutic proteins. A fraction of the patients treated with those

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immunogenicity is typically related with vaccines where the injection of an antigen (the vaccines) has to lead to an immune response against the pathogen. Unwanted immunogenicity is when organism mounts an

METHODOLOGY

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	Autotransporter beta-domain; Secretion of protein products occurs by a number of different pathways in bacteria. One of these pathways known as the type V pathway was first described for the IgA1 protease. The protein component that mediates secretion through the outer membrane is contained within the secreted protein itself, hence the proteins secreted in this way are called autotransporters. This family corresponds to the presumed integral membrane beta-barrel domain that transports the protein. This domain is found at the C terminus of the proteins it occurs in the variable passenger domain that is translocated across the membrane. Once the passenger domain is exported it is cleaved auto-catalytically in some proteins, in others a different protease is used and in some cases no cleavage occurs.	
	- Pssm-ID: 252169 Cd Length: 268 Bit Score: 75 22 E-value: 8.08e-15	
	10 20 30 40 50 60 70 80	
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	gi 23506944 1139 DQAQSAVWIN-IAQDKRRYDSDAFRAYQQKINLRQIGVQHAL-DNGRIGAVESHSRSDNIFDE-QVKNHATLANMSGFAQ 1215 Cdd:pfamU3797 1 EPEDNGVWARgLGGFGHQDS3GESAGFRSRSGGYQLGADARLqGDLILGLAFGYSFSKSKFDDqGGKGKSDSYGAGLYAQ 80	
	90 100 110 120 130 140 150 160	
	gi 23506944 1216 YQWEDIQFGVNVGAGISASKMAEEQSRKIHRKAINYGVNASYQFRLGQ-LGIQPYLGVNRYFIERENYQ 1283 Cdd:pfam03797 81 WNLDC-CLYLDGVLAYGRFDMdvkrsvdlg:1SDTAKCDVDSHCLGASLEAGYRFKLSGaLTLT9FAGLQVVLRQDGFT 159	
	odurgramovra – oz nazod ozrzedniczen znanagyzerznegzennegzennegniczen zwierzegy rzegyerz ros	
	170 180 190 200 210 220	
	gi 23506944 1284SEEVKVQTPSLVENRYNLGIEVTNYFtptDNISIKPYFFVNYVDVSNANVQTTVNRTMLQQS 1345	
	<pre>gi Xisbosys 1226ELEVENGIPELVERIGEELEVENTETETETETETETETETETETETETETETETETETE</pre>	
	+) Peptidase S6 pfam02395 Immunoglobulin A1 protease; This family consists of immunoglobulin A1 protease proteins. The 26-767 0e+00	
	+) Trichoplein plam13868 tumor suppressor, Mitostatin, Trichoplein or mitostatin, was first defined as a 983-1172 6.64e-06	
	References:	
	Warchler-Bauer A et al. (2011), "CDD: a Conserved Domain Database for the functional annotation of proteins.", Nucleic Acids Res.39(D)225-9.	
	Warchler-Bauer A et al. (2009), "CDD: specific functional annotation with the Conserved Domain Database.", Nucleic Acids Res.37(D)205-10.	
	W Marchler-Bauer A, Bryant SH (2004), "CD-Search: protein domain annotations on the fly: ", Nucleic Acids Res.32(W)327-331.	
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-			Pssm-ID: 2612	69 (Multi-doma	in] Cd Lengt	h: 759 Bit Sc	ore: 757.03	E-value: 0e+	DO		
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	Cdd:pfam02395	80 SVSFGNYGNN	TYTIVDRI	INYPPLD	FHMFRLNKF	VTEVAPÄAVI	Angsvagay	DRERYPVFVI	LGSGRQ 148		
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	gi 23506944 763 TLSNN 767 Cddipfam02395 755 TLSGS 759
	H Trichoplein pfam13868 tumor suppressor, Mitostatin, Trichoplein or mitostatin, was first defined as a 983-1172 6.64e-06
	tumor suppressor, Mitostatin, Trichoplein or mitostatin, was first defined as a meiosis-specific nuclear structural protein. It has since been linked with mitochondrial movement. It is associated with the mitochondrial outer membrane, and over-expression leads to reduction in mitochondrial motility whereas lack of it enhances mitochondrial movement. The activity appears to be mediated through binding the mutochondria to the actin intermediate filaments (IF's).
	Pssm-ID: 258135 [Multi-domain] Cd Length: 350 Bit Score: 48.37 E-value: 6.64e-06
	10 20 30 40 50 60 70 80
	gi 23506944 983 EQELESDLVAAEQAXXIIAAQUVQTAATQTSXIRVESERAVYSDDipAQSLLKA
	90 100 110 120 130 140 150 160
	gi 23596944 1039 EARQALTIETGTSKARKVISKRAAREfedtlpdGILQAALEVIDAQQVKKEDGTQELEERRQKKQKELIStyenealSE 1118 Cdd:pfam13069 121 EKQKRIREDIDEFWEERIWKTEEKEREREEEKLIEYGREKAEREEERFAERAERKEEKEFVAR 186
	170 180 190 200 210
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	g1 23506944 1119 LSATVNSHLSVQDELDRLFVDQAQSAvvcHIAQDKRRYDSDAFRAYQQETHLAQ 1172 Cdd:pIam13865 187 LRAQQEEAEDEREELDELRADLYGEEYERKERQKKEEAEKRRQKQELQR 237
	References: Marchier-Bauer A et al. (2011), "CDD: a Conserved Domain Database for the functional annotation of proteins.", Nucleic Acids Res. 39(D):225-9.
	Wharchier-Bauer A et al. (2011), CDD: specific functional annotation with the Conserved Domain Database.", Nucleic Acids Res.37(D)205-10.
	Marchier-Bauer A, Bryant SH (2004), "CD-Search: protein domain annotations on the fly,", Nucleic Acids Res.37(D)205-10.
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3. Secondary structure Prediction

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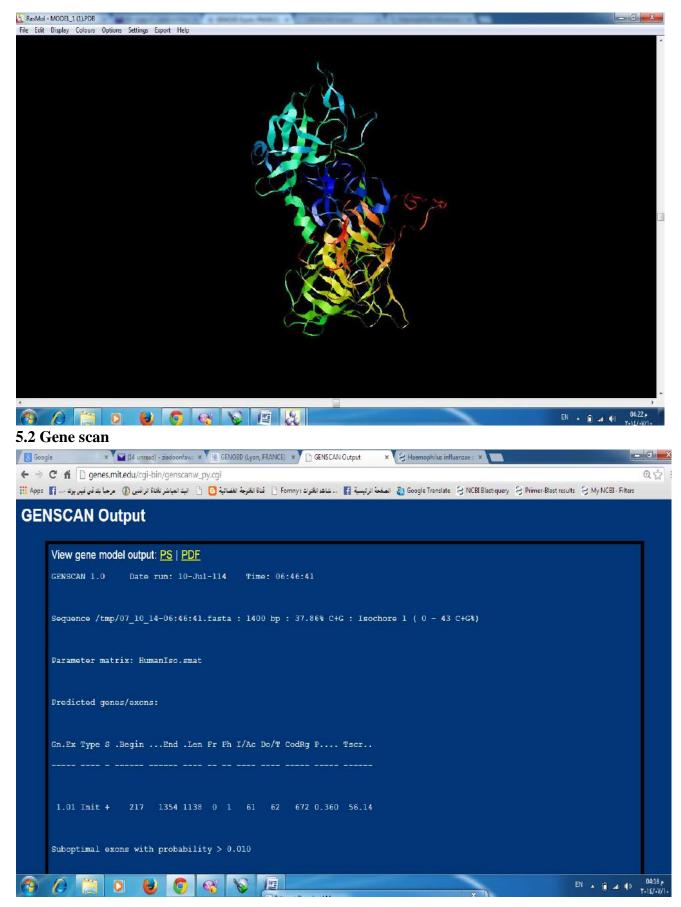
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4. Functional analysis

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31 <u>0</u> 32 <u>0</u> AMEHGKNI YFGDR <mark>GT</mark> GTL TIENI	33 <u>0</u> 34 <u>0</u> 35 <u>0</u> NINGGA GGLYFEGNFT VSSKNNATWQ GAGV	36 <u>0</u> HVSEDS			
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5.1 Geno 3D





5.3 Gene mark

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Masking: default					
Masked variables: [1, 2, '	cterm']				
Predictions:					
Peptide 🔹 🔹	Length +	Score •			
FYTEVLAVDIPSVFRRYIPSINGHYSF	27	0.40462			
SPRFIND	7	0.18692			
AFSEIGLVSG	10	0.13739			
GVATLVGDQYIV5VAH	16	0.09113			
GAYNYLIA	8	0.09057			
KGTLHVK	7	0.0718			
QGAGVHVSE	9	0.05563			
AKSEVGIVKLFN	12	0.05429			
FINGVLQTGHPF	12	0.03912			
GIVHESGN	8	0.03282			
HMPRLHKFVID	11	0.0269			
HYGPLPI	7	-0.0214			
HRFTYQI	7	-0.03558			
MIDESVVSR	9	-0.05906			
GSISVGDGKVILE	13	-0.06077			
DLNGHSLTF	9	-0.07891			
AYSSYDI	7	-0.20289			
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6. A. Phylogenic analysis (DNA sequence)

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	genetic tree.						3

6. B. Phylogenic analysis (Protein sequence)

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7. A. Immunogenicity prediction (Hap protein)

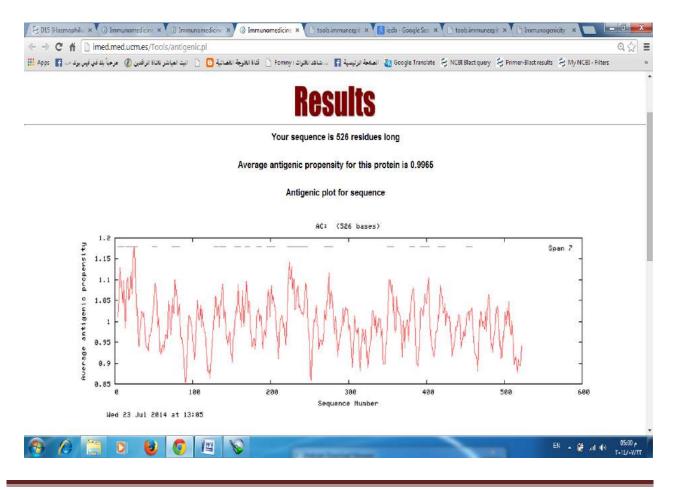
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MHC-I Binding Prediction Results

	Name	Sequence
1	sequence 1	ICAPILDESVVSINGVATIVGDQITVSVAINOSYNSVUDDAEORYEDGER TIQTVBDVYKEGONAPBOTANERLEKETTÖLEEIGETIMOGOVAN SUDFERFILSSERVYNETOHOETINSYSDIGSVAINTIANETTEGS CONSTVERSINGVERSUSSERVITVSSUDGENETINSY UDGFBCNILSSERVYNETOHOETINSYSDIGSVAINTIANETTEGS UDGFBCNILSSERVYNETOHOESUSSERVITVEINETSINGPOST UDGFBCNILLIRENDESKASEUSTVELENDIGENGITTYSSEILLANG GAVTTYSDIGTTERVITVEIDALAKCENTIANEADERSING GAVTTYSDIGTTERVITVEIDALAKCENTIANEADERSINGUSSE UDMORGALLIRENDIGENGUSSENVILTYVAINTEGVANTETYSSEILLANG GAVTTYSDIGTTERVITVEIDALAKCENTIANEADERSISTANG LINGKSLIFFOLGINGESELDIGNORVULLDIANEGTUSKIPTSEOLOM LUNGSLIFFOLGINGESELDIGNORVULLDIANEGTUSKIPTGVAINTET UDMORGINGESELDIGNORU

Prediction method: IEDB recommended | Low percentile_rank = good binders
Download result

Allele 💡	**	Slart 🛊	End	Length	Peptide #	Method used	Percentile rank	ANN IC50(nM)	ANN renk ÷	SMIM IC50(nM)	SMM rank +	Comblib_Sidney2008 score	Comblib_Sidney2008 rank
HLA- A*02:01	1	225	233	9	VLAVDIPSV	Consensus (ann/comblb_sidncy2008/smm)	0.4	9	0.4	27.38	0.5	68-06	0.4
HLA- A*02:01	1	199	207	9	VLQTCHPFV	Consensus (ann/comblib_sidney2008/smm)	0.8	19	8.0	48.10	0.8	2.13e-05	13
HLA. A*02:01	1	17	25	9	TINCDONIA	Consensus (ann/combilib_sidney2008/smm)	1.5	50	1.6	62.97	1.2	2.54e-05	1.5
HLA- A=02:01	1	139	147	9	YLIAGNIHT	Consensus (ann/combib_sidney2008/smm)	1.8	53	1,7	111.97	1.8	0.000111	6.2
HLA- A#02:01	1	333	341	9	LYFEGNETV	Consensus (ann/combib_sidney2008/smm)	2.6	141	2.6	117.52	1.8	4.908-05	2.8
HLA- A*02:01	1	240	248	9	SINGHYSFV	Consensus (ann/comblb_sidney2008/amm)	3.7	107	2.3	311.97	3.7	0.000318	16
HLA- A*02:01	4	378	384	9	KIGKGILHV	Consensus (ann/comblib_sidney2008/smm)	4	228	3.1	351.65	4	0.000385	19
HLA- A*02:01	1	1	9	9	RAPMIDESV	Consensus (ann/combile_sidney2008/smm)	4.8	512	4.2	500.16	4.8	0 000896	32



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8. A. Immunogenicity prediction (D15 protein)

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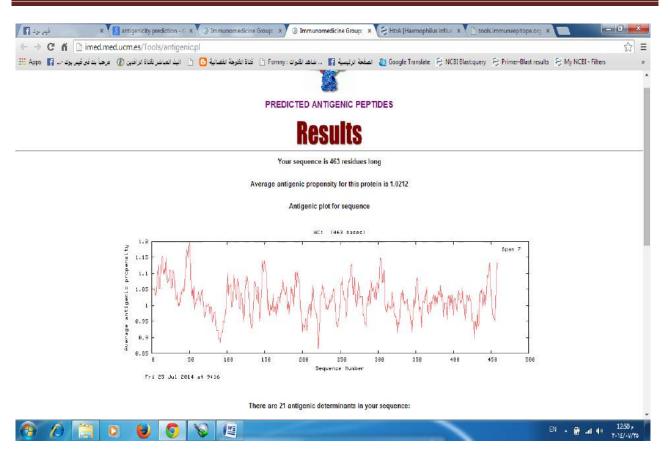
Predictions:

Peptide 🔹	Length #	Score •
ASAELIVPTPFVS	13	0.41193
AITFVVDA	9	0.28292
ITVFAAPFVAKD	12	0 25648
WGDVLIR	8	0.1754
ANIVRSLEVS	11	0.1738
GGRVTI	7	0 13982
RLTVRQ	7	0.12438
DEVDVVYK	8	0.11166
IKVNVII	7	0.08363
SYYVGL	7	0.06718
RTSLEVDAAS	11	0.06629
QYDLRS	7	0.05904
AKSVKEHYASVGRYNATVEPIVN	24	0.04943
GSNVTLGF	9	0.02697
KGNSVIPTE	10	0.02219
FEKDLQAIRD	10	0.00811
LPFYQT	7	0.00156

8. B. Immunogenicity prediction (D15 protein)

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dictior	YI SI VI AS	KLSADVÇ LRGFAYO SDKSQNT STGVGPÇ thod: II	GFYPLD SIGPNA VRTSLF WQSPIG	RDHRWVVS IYAEHGNO VDAASVWS IPLVFSYAE	TFNKISSDVI TKWKSDKNGI PIKKYENDDV	FREMERLEFFQITTAGGIG IGGNAITTASAELIVPTPF LESKVIKULEPYCKSSBIR /EQPQFBIGSF	d binders	S							
wnload ations	n met	KLSADVÇ LRGFAYO SDKSQNT STGVGPÇ thod: II	GFYPLD SIGPNA WRTSLF WQSPIG	RDHRWVVS IYAEHGNO VDAASVWS IPLVFSYAE	TFNKISSDVI TKWKSDKNGI PIKKYENDDV	IGGNAITTASÄELIVPTPF LESKVLKDLPDYGKSSRIR VEQPQFSIGGSF	d binders	Percentile	ANN	AHN	SMM	SMM	Comblib_Sidney2008	Comblib_Si	dney2008
wnload ations ck to exp	n met	KLSADVÇ LRGFAYG SDKSQNT STOVGPÇ thod: II t II	IGFYPLD SIGPNA VRTSLF WQSPIG EDB re	RDHRWVVS IYAEHGNG VDAASVWA PLVPSYAP	TENKISSOVI Itkniksoknez IPIKKYENDOV Ided Low Peptide	IGGNATITASALITYPTPF IESAVIALIOPOKEKSBIR PEQFQFSIGSSF percentile_rank = goo	\$		ANN IC50(nM) 10	ANN Fank 0.5	SMM IC50(nM) 18.67:	SMMM rank	Comblib_Sidney2008 Score 1,18e-05	rai	dney2008 Ik 8
wnload ations ck to exj Jlele HLA-	n met result	KLSADVÇ LRGFAYG SDKSQNT STGVGPÇ thod: II t I	GFYPLD SIGPNA VRTSLF WQSPIG EDB re	RDHRWVVS IYARHGNO VDALSVNN PLVFSYAP	TERNELSSOUL TRANSSOUNCE PEREVENDEN Ided Low Peptide	IGGKATITASÄELIYVPTPF LESKVIKLIOPKEKSERIE PEQFQFSIGSSF percentile_rank = goo Method used Consensus	* m)	Percentile	IC50(nM)	rank	IC50(nM)	rank	score	* ran 0	k
wnload ations ck to exj liele HLA- 102:01 HLA-	result pander	KLSADVQ LRGFAYG SDKSQNT STGVGFQ thod: If t Start Start 9	IGFYPLD SIGPNA VRTSLF WQSPIG EDB re	RDHRWVVE II YAA HGMC VDAJSVNP PLVPSYAP COMMER	TERNESSDVI ITKWESDKNSI PERVENDU Ided Low Peptide LLFOTTIV KTLAITEVV	IGGNATITASÄELIYPTPF LESKVIKULEDVGKSSRIR TEQFQFSIGBSP percentile_rank = goo Method used Consensus (ann/combilib_sidne/2008/sm Consensus	* im) im)	Percentile rank 0.5	KC50(nM) 10	rank 0.5	IC50(nM) 18.67	rank 0.4	score 1.16e-05	• Tan 0 0	1k .8
wnload ations ck to exp Jiele HLA- *02:01 HLA- *02:01 HLA-	yrr SF VS As result pander 1 1	KLSADVQ LRGFAYG SDKSQNT STGVGFQ thod: II t Start 9 330	IGFYPLD SIGPNA VRTSLF WQSPIG EDB re IN End 17 338	RDHRWVYS IYAR HGNG VDALSVM IPLVFSYAB COMMER 9 9	TENKISSDVI TENKISSDVI TENKISDVIST PERKYENDOV Ided Low Peptide LLF0TTTV KTLAITEVV SLFVDAASV	IGGKATTTASÄELTYPTPF LESKYTKELDAVGKSSBTR PEQPQFSIGSSF percentile_rank = goo Method used Consensus (annvcombilb_sidne/2008/sm Consensus (annvcombilb_sidne/2008/sm Consensus	(m) (m)	Percentile rank 0.5 0.9	10 10	rank • 0.5 0.5	IC50(nM) 18.67 59.86	rank •	1.16e-05	• Fair 0 0 0	1 k 18 1.9
wnload ations ck to exp Jele HLA- 102:01 HLA- 102:01 HLA- 102:01 HLA-	yr Si Vy 2 3 3 7 result cander	KLSADVC RGFAYG SDKSQNT STGVOFC thod: If t d the resu Start 9 330 712	IGFYPLD SIGPNA VRTSLF WQSPIC EDB re Int Int Int Int Int Int Int Int Int Int	RDRRWYS LIYAEHGRO VDALSVIN PLVPSYAP COMMER 9 9 9 9	TENKI SSDVI TENKI SSDVI PIKYENDOV Ided Low Peptide LLFETTETV KTLAITEVY SLEVDASV KLIEFAKSV	IGGKATTTASAELTYPTPF LEXXVIXEDOVGKSSBIR TEQFQ7316857 percentile_rank = goo Method used Consensus (ann/combile_sidne/2008/sn Consensus (ann/combile_sidne/2008/sn Consensus (ann/combile_sidne/2008/sn Consensus Consensus	* im) im) im)	Percentile rank 0.5 0.9 1	10 10 26	rank 0.5 0.5 1	18.67 59.86 31.20	1.2	1.16e-05 1.39e-05 2.88e-05	0 0 1 2	nk 1.8 1.9 .7

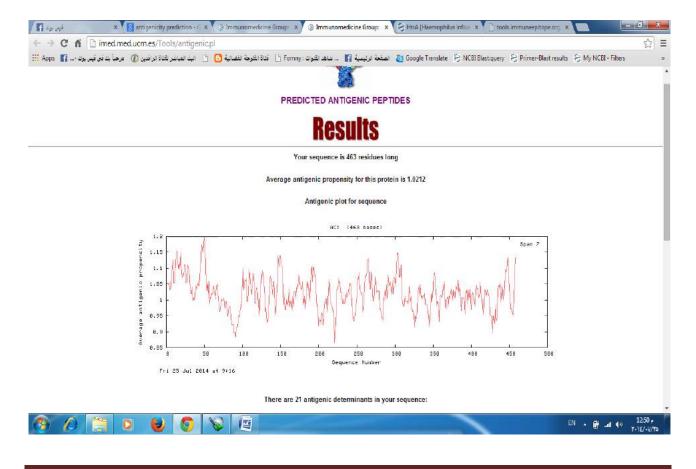


9. A.Immunogenicity prediction (Htra protein)

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Masking: default									
Masked variables: [1, 2, 'cter	m'1								
Predictions:	1								
Peptide +	Lengthe	Score							
DKLRVGDFTVAI	12	0.35152							
GINTAIIS	8	0.31266							
SIAFAIPS	8	0.28452							
KISSFAEIRA	10	0.16874							
LG5GVII	7	0.14588							
SDIALVQL	9	0.13594							
SKIELPALDG	10	0.13218							
SKGYVLIN	8	0.0698							
LETEPSAVALNI	12	0.04344							
BGALVNL	7	0.03565							
REGLIG	7	0.02966							
DHITVQL	7	0.01342							
VISGIVSA	8	0.00924							
KAKLVGK	7	-0.00906							
ASNLVQQILEF	11	-0.02424							
KSHDVRM	7	-0.12998							
NADLAHAFNVSAQQGAFVSEVLPH	24	-0.15374							
AHVDSRSPFLD	11	-0.1542							
<u>genslaag</u> r	9	-0.1772							
NSLAPMLEKVQPAVVTLSVEG	21	-0.26962							
IRFVLNSIALGLSVLSTSFVAQAILPSFVSE	31	-0.31458							

9. B

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HC	-I E	Bind	ing l	Predi	ction F	Results								
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		SGVIII VQLEK TGSDS IAFAI AFVSE SLTYL EITKI	NASKGYV PSNLTEI STYENYI PSNQASN VLPKSAA RDGRSHI QPNSLAA	LTNNHVII KFADSDKI QTDAAVNI ILVQQILEI LERAGLKA(WENKLQAI LQRGLESGI	DEADKITVQLQ LRVGDFTVAIO RGNSGGALVNI FGQVRRGLLGI SDIITAMNGQF DD83QL88KTE	DRFAEQPGGROESERNFROLG 2DGREFKARLVGKDELSDIAL NMPFGLGGVT3GIVSALGRS NGELIGINTATISPSGGNAG KKGGELINADLATAFNVSAQQG LISSFAFLBARLATFGAGKEI 2LPALDGATLKDYDARGVKGI LENIRELNKVLDTEPSAVALN								
wnload	d res				nended Lo	ow percentile_rank = go	ood binders							
wnload ations ack to ex	d res	nethod	: IEDB		nended Lo Peptide	ow percentile_rank = go Method used	Percentile	ANN 1(550mM)	ANN	SMM	SMM	Comblib_Sidney2008	Comblib_Sidn	iey2008
wnload ations ack to ex	d res	nethod sult 🗷 ded the n	: IEDB	recomn	S			ANN IC50(nM) 16	ANN rank 0.7	SMM IC50(nM) 48.99	SIMM rank 0.8			1ey2008
wnload ations eck to ex lele HLA- 102:01 HLA-	d res	tethod sult 🗷 ded the n	: IEDB esult 🕑 End 🛊	recomn	Peptide	Method used	Percentile rank	IC50(nM)	rank	IC50(nM)	rank	score	rank	
wnload ations eck to ex liele HLA-	d res	tethod sult 🛎 ded the n Start 37	: IEDB esult End 45	recomn	Peptide	Method used Consensus (ann/comblib_sidney2008/smm) Consensus	Percentife rank 0.7	IC50(nM) 16	rank = 0.7	1650(nM) 48.99	rank C.8	score 2.4e-06	Tank 0.2	
HLA- 102:01 HLA- 102:01 HLA- 102:01 HLA- 102:01 HLA-	xpanc 1	sult 🗷 ded the ro Start 37 41	End 45	Length 9 9	Peptide SLAPMLERV MLEKVQPAV	Method used Consensus (ann/combilib_sidney/2008/smm) Consensus (ann/combilib_sidney/2008/smm) Consensus	Percentife rank 0.7 1.3	1C50(nM) *	0.7	48.99 88.13	rank • 0.8 1.5	2.4e-06	Tank 0.2 1.2	
wnload ations eck to extra to extr	xpanc # 1 1	ded the ro Start 37 41 66	: IEDB esult End 45 49 74	Length 9 9	Peptide SLAPMLEKV MLEKVQPAV FLDDIPEEF	Method used Consensus (ann/comblib_sidney2008/smm) Consensus (ann/comblib_sidney2008/smm) Consensus Consensus Consensus Consensus	Percentile rank 0.7 1.3 1.9	1C50(nM) 16 38 122	rank 0.7 1.3 2.5	48.99 88.13 119.15	rank • 0.8 1.5 1.9	2.4e-06 1.96e-05 7.69e-06	• Fank 0.2 1.2 0.5	



RESULT

Generalized study of sequence and structure comparison studies, To find out which is the best disease causing target antigens we perform epitope prediction for binding site analysis among the predicted antigens which is showing best antigenicity and immunogenicity score, basing on propensity values for antigenicity and Immunogenicity values and ranking of immunogenicity considered, we select the best targets of HPV type b strains. Among the all selected antigens, Hap, HtrA 1.0024 are showing best Antigencity and also showing best Immunogenecity Hap (immunogencity score(IM score) 0.64 for 48 residues ; P-value -1.22023e).

CONCLUSION

Cancer vaccines in cancer therapies is called immunotherapy which is done either by specific cancer vaccine or universal cancer vaccine which contain tumor antigens that stimulate the immune system which in turn initiate various mechanisms that terminate tumor cells and prevents recurrence of these tumors. Here best antigens are identified these target antigens may helpful for further studies and there may be scope to develop new drugs which cal bitterly interact with selected targets. In these studies finally three best targets are identified as specified in result part.

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CONFLICT OF INTEREST

The authors report no conflict of interest.

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