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In-Silico Prediction of T- cell epitopes in VacA: First Step towards the Design of a *Helicobacter Pylori* Vaccine

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ABSTRACT

Helicobacter pylori are indicated in gastritis, gastric cancer, Peptic ulcer and B cell mucosa lymphoid tissue lymphoma. The bacteria have a 50% pandemic, a higher prevalence of 75% in developing countries and 40% in developed countries. A combination of antibiotics is used to treat Helicobacter pylori infections. Some of these are Amoxicillin, tetracycline, and fluoroquinolones, but are ineffective in eliminating the infections and are also compromised by antibiotic-resistant strains, therefore a vaccine to help prevent, control and eliminate Helicobacter pylori infections is needed. We proposed to predict T-cell epitopes of VacA. Seven VacA protein sequences were retrieved from the NCBI protein database and subjected to antigenicity test using the ANTIGENpro database. All the seven (7) sequences with antigenicity score ≥0.8 were submitted to the NetCTL server and IEDB (Immune Epitope Database) MHC-II server to predict the cytotoxic T-lymphocyte (CTL) and to predict the Helper T-lymphocyte (HTL) epitopes, respectively. The cytotoxic T-lymphocyte epitopes and Helper T-lymphocyte epitopes were predicted. The seven amino acid sequences obtained from NCBI subjected to antigenicity test passed by having a score of ≥0.8. Ninety-eight (98) CTL (9-mer) ligands in total were predicted for the seven selected proteins submitted to NetCTL sever. Fifty-six (56) epitopes were selected according to their high scores. Ninety-nine (99) (15-mer) total HTL epitopes were predicted. Their IC50 value was not greater than 500nm with percentile rank ranging from 0.030-4.00, while ninety-four (94) epitopes were selected. A total of fifty-six (56) CTL and Ninety-four (94) HTL epitopes were merged using AAY, EAAK, and GPGPG linkers with sequence APPHALS as an adjuvant to construct the multi-epitope vaccine. The predicted peptides are presumed to be valuable in the multi-epitope vaccines, non-allergen without compromising the population coverage. VacA has potential B-cell and T-cell epitopes distributed throughout the sequence. Thus several fragments were identified as valuable candidates for subunit vaccines against Helicobacter pylori.

Keywords: *Helicobacter pylori*, Peptic Ulcer, Vaccine, Gastric cancer, VacA

INTRODUCTION

Helicobacter pylori are gram-negative, microaerophilic bacteria. They are spiral shaped flagellates and populate the human gastric mucus membrane [1]. *H. pylori* colonization typically does not trigger clinical presentation but can increase the risk of developing gastrointestinal ulcer diseases, lymphoma, and gastric cancer. *H. pylori* infections in many patients are asymptomatic, and only a few people develop clinical manifestations [2]. *Helicobacter pylori* are responsible for Gastritis, Gastric cancer, Peptic ulcer, B cell mucosa lymphoid tissue lymphoma [3]. The bacteria are 50% pandemic and have a higher prevalence of 75% in developing countries but, in developed countries, prevalence is 40% [1]. The virulence factor of *Helicobacter pylori* are vacA, cagA, dupA, oipA, and s1m1. Infection by *H. pylori* with positive vacA, s1m1and the cagA genes increases the possibility of developing gastric cancer. The correlation between vacA, s1m1, and the cagA and gastric cancer indicates that screening of these genes may be beneficial for detecting populations at greater risk for gastric cancer [4,5]. Co-expressing *H. pylori* virulence factors can predict severe *H. pylori* infection, like a peptic ulcer or gastric cancer. Thus far, several new virulence factors of *H. pylori* have been depicted; nonetheless, an established correlation between these new virulence factors and gastric cancer has not been presented; however, it has been projected that specific analysis of *H. pylori* virulence factors can be pertinent for predicting post-*H. Pylori* infection disorders [6,7]. Over the years,

Helicobacter pylori infections have been treated with a combination of antibiotics, some of which are Amoxicillin, tetracycline, and fluoroquinolones but are not effective enough to eliminate the infection and are also compromised by antibiotic-resistant strains [8] hence a vaccine to help prevent, control and eliminate *Helicobacter pylori* infections is needed. Epidemiological surveys indicate that up to 90% of gastric cancer could be attached to *Helicobacter pylori* infection and that elimination of this pathogen could relegate the prevalence of gastric cancer. Screening for and suppression of *H. pylori* is consequently an insinuating approach to eradicate gastric cancer. This study suggests the design of a multi-epitope vaccine against Helicobacter pylori infections.

MATERIALS AND METHODS

Retrieval of protein sequences and antigenicity testing

Seven VacA protein sequences were retrieved in FASTA format with accession number AAU85846.1, ADPO2392.1, ADPO2391.1, ADPO2390.1, ADK63389.1, ADK63388.1, and ADK63387.1 from the NCBI protein database after which their antigenicity was predicted using the **ANTIGENpro** database (http://scratch.proteomics.ics.uci.edu/). All the protein sequences selected obtained a probability for antigenicity score of ≥ 0.8 [9].

Prediction of CTL Epitopes

All the seven (7) sequences with antigenicity score ≥ 0.8 were subjected to **NetCTL** (http://www.cbs.dtu.dk/services/NetCTL/) in FASTA format. The cytotoxic T-lymphocyte epitope was predicted at the default threshold score of 0.75, those with a combined score >0.75 were further selected as CTL epitopes and **IEDB MHC I** immunogenicity model was used to predict their **HTL** epitopes [9].

Prediction of HTL Epitopes

VacA protein sequences retrieved from NCBI server are subjected to Immune Epitope Database (IEDB) MHC-II epitope prediction module (http://www.iedb.org/). The selected alleles for prediction are H2-IAb, H2-IAd and H2-IEd mouse alleles, while other parameters were left default.

Multi-epitope subunit vaccine sequence construction

A total of 56 CTL and 94 HTL epitopes were merged together using AAY and GPGPG linkers. AAY linker was used to bind intra-CTL epitopes and a GPGP linker to bind intra-HTL epitopes while using a TLR-4 agonist with sequence APPHALS as an adjuvant. APPHALS was joined to the CTL and HTL epitopes by EAAY linkers.

Vaccine allergenicity and antigenicity prediction

Antigenicity of the constructed multi-epitope subunit vaccine was predicted using ANTIGENpro sever (http://scratch.proteomics.ics.uci.edu). ANTIGENpro utilizes protein antigenicity microarray data to predict protein antigenicity. This server is an alignment-free and pathogen-independent predictor that is sequence-based [9] while AllerTOP v. 2.0 (https://www.ddg-pharmfac.net/AllerTOP/) online servers was used to predict the allergenicity.

Determination of physiochemical properties

Physiochemical properties such as amino acid composition, theoretical pI, instability index, in vitro and in vivo half-life, aliphatic index and molecular weight were predicted with ProtParam sever (http://web.expasy.org/protparam/).

RESULTS

Retrieval of protein sequences and antigenicity testing

Table 1 shows the seven amino acid sequences with accession numbers AAU85846.1, ADPO2392.1, ADPO2391.1, ADPO2390.1, ADK63389.1, ADK63388.1, and ADK63387.1 obtained from the NCBI database in FASTA format, which was selected according to their antigenicity score obtained from the ANTIGENpro server. All the proteins have a score of ≥ 0.8 [9].

Table 1: Retrieval of *Helicobacter pylori* VacA protein sequences for vaccine construct (from the NCBI database and their antigenicity scores)

S/N	Protein Accession No.	Antigenicity Score	Selected/Non Selected
1	AAU85846.1	0.9590	Selected
2	ADPO2392.1	0.9581	Selected
3	ADPO2391.1	0.9581	Selected
4	ADPO2390.1	0.9557	Selected
5	ADK63389.1	0.8728	Selected
6	ADK63388.1	0.8983	Selected
7	ADK63387.1	0.9126	Selected

Cytotoxic T-lymphocyte (CTL) Epitope Prediction and Immunogenicity Assessment

98 CTL (9-mer) ligands in total were predicted for the seven selected proteins, and 56 epitopes were selected according to their high scores, as shown in Table 2.

Table 2: Cytotoxic T-Lymphocytes (CTL) prediction and immunogenicity (NetCTL1.2)

S/N	Protein Accession No.	Epitopes	Comb. score	Length	IEDB immunogenicity score	Selected/Non-selected
1	AAU85846.1	DMKDAVGTY	0.9988	9	0.08056	Selected
		SKNAEISLY	0.9016	9	0.11395	Selected
		YSTINTSKV	0.8096	9	-0.08886	Non-selected
		NTSKVTGEV	1.1167	9	-0.10686	Selected
		KTEIQPTQV	0.7704	9	0.00826	Selected
		LTTNAAHLH	0.8141	9	0.10184	Selected
		TTNAAHLHI	0.9979	9	0.11536	Non-selected
		RVNNQVGGY	1.2065	9	-0.03415	Non-selected
		GTDTKNGTA	1.3160	9	-0.11855	Selected
		GTATFNNDI	0.9499	9	0.16717	Selected
		KVDAHTANF	0.8680	9	0.14385	Selected
		GIDTGNGGF	0.8486	9	0.12157	Non-selected
		VTDKVNINK	1.3597	9	-0.06715	Non-selected
		YSQFSNLTI	0.9710	9	-0.07367	Selected
		DIDSATGFY	2.9331	9	0.01237	Selected
		LLKAKIIGY	0.7576	9	0.01677	Selected
2	ADPO2392.1	DMKDAVGTY	0.9989	9	0.08056	Selected
		ITSDKNAEI	0.7744	9	-0.15595	Non-selected
		TSDKNAEIS	0.7895	9	-0.01701	Non-selected
		YSTINTSKV	0.8096	9	-0.08886	Non-selected
		NTSKVTGEV	1.1167	9	-0.10686	Non-selected
		QSSNSNTEV	1.2607	9	-0.13614	Selected
		KTEIQPTQV	0.7620	9	0.00826	Selected
		TTNAAHLNI	0.7918	9	0.09268	Selected
		RVNNQVGGY	1.2088	9	0.12908	Selected
		GTATFNNDI	0.9499	9	0.16717	Selected
		KVDAHTANF	0.8680	9	0.14385	Non- selected
		GIDTGNGGF	0.8486	9	0.12157	Selected
		YSQFSNLTI	0.9710	9	-0.07367	Selected
		DIDSATGFY	2.9331	9	0.01237	Selected
		LLKAKIIGY	0.7576	9	0.01677	Non-selected
		SMVNNPDNY	0.8944	9	0.00509	Non-selected
		RSKDIDTLY	1.9109	9	0.12908	Non-selected
		LIDSHDAGY	2.4031	9	-0.05407	Non-selected
		ATDALNNVA	1.2167	9	0.04834	Non-selected

3	ADPO2391.1	DMKDAVGTY	0.9989	9	0.08056	Selected
		YSTINTSKV	0.8283	9	-0.08886	Non-selected
		NTSKVAGEV	1.0111	9	-0.10657	Non-selected
		KTEIQPTQV	0.7686	9	0.00826	Selected
		LTTNAAHLH	0.8141	9	0.10184	Selected
		TTNAAHLHI	0.9979	9	0.11536	Non-selected
		MVNNQVSGY	1.7462	9	-0.20237	Selected
		GTDTNNGTI	1.9771	9	0.08515	Selected
		DTNNGTIAF	0.8203	9	0.19611	Selected
		DIDTGNGGF	0.8367	9	0.12157	Selected
		KLVINDFYY	0.7683	9	0.25854	Selected
		LLKAKIIGY	0.7572	9	0.01677	Selected
		SMVNNPDNY	0.8944	9	0.00509	Selected
		RSSDIDTLY	3.0555	9	0.14538	Selected
		SSDIDTLYA	1.4235	9	0.18774	Non-selected
		LIDSHNAGY	2.8021	9	-0.08104	Selected
		NIDSFAQRL	0.7645	9	-0.07596	Selected
		GTSAGVDAY	2.6876	9	0.09911	Non-selected
		GSDQSSLNF	2.3621	9	0.08515	Non-selected
		LLRDLNQSY	0.9202	9	-0.17219	Selected
		NQSYNYLAY	1.9312	9	-0.0537	Non-selected
		YSAATRASY	2.5136	9	0.07495	Non-selected
		AATRASYGY	0.8299	9	-0.03627	Non-selected
		ASANVEARY	2.1570	9	0.2039	Non-selected
		SANVEARYY	1.0905	9	0.21529	Non-selected
		YYGDTSYFY	1.0136	9	-0.01933	Non-selected
		FASNLGMRY	2.4986	9	-0.15707	Non-selected
4	ADPO2390.1	DMKDAVGTY	0.9989	9	0.08056	Selected
		YSTINTSKV	0.8096	9	-0.08886	Non-selected
		NTSKVTGEV	1.1167	9	-0.10686	Non-selected
		KTEIQPTQV	0.7686	9	0.00826	Non-selected
		TTNAANLNI	0.8941	9	0.05614	Non-selected
		MVNNQVGGY	1.7930	9	-0.03415	Non-selected
		GVDTKNGTI	0.8543	9	-0.11855	Non-selected
		DIDTGNGGF	0.8367	9	0.12157	Non-selected
		KLVINDFYY	0.7683	9	0.25854	Non-selected
		YSQFSNLTI	0.9567	9	-0.07367	Non-selected
		NVDSATGFY	3.2718	9	0.01237	Non-selected
		LLKAKIIGY	0.7572	9	0.01677	Non-selected
		SMVNNPDNY	0.8944	9	0.00509	Non-selected
		TSNARFASY	2.2171	9	0.13423	Non-selected
		RSSDIDTLY	3.0554	9	0.14538	Non-selected
		SSDIDTLY	1.4101	9	0.18774	Non-selected
		LIDSHDAGY	2.4054	9	-0.05407	Non-selected
		NSGGNTSLY	2.4453	9	-0.07076	Non-selected
		GTSAGVDAY	2.6964	9	0.09911	Non-selected
		GSDQSSLNF	2.3621	9	-0.43933	Non-selected
		LLRDLNQSY	0.9213	9	-0.17219	Non-selected
		NQSYNYLSY	1.7050	9	-0.17322	Non-selected
		ASTRASYGY	1.4402	9	-0.03627	Non-selected
		ASANVEARY	2.1570	9	0.2039	Non-selected
		SANVEARYY	1.0905	9	0.21529	Non-selected
		YYGDTSYFY	1.0136	9	-0.01933	Non-selected
		FASNLGMRY	2.4986	9	-0.15707	Non-selected
5	ADK63389.1	DMKDAVGTY	0.9988	9	0.08056	Selected
		ITSDKNAEI	0.7744	9	-0.15595	Non-selected
		TSDKNAEIS	0.7895	9	-0.01701	Non-selected
6	ADK63388.1	DMKDAVGTY	0.9988	9	0.08056	Selected
7	ADK63387.1	DMKDAVGTY	0.9989	9	0.08056	Selected

Helper T-lymphocyte (HTL) Epitope Prediction

IEDB MHC-II prediction tool was further used to predict the MHC-II binding molecules of the six epitopes. A total of 27 HTL epitopes were predicted. Their IC_{50} value ranging from 1284 – 5366 with percentile rank ranging from 15-16 (Table 3).

Table 3: Helper T-Lymphocytes (HTL) epitope prediction and immunogenicity and their percentile ranks for Helicobacter pylori as obtained from the immune epitope database (IEDB)

Seq. no	Alelle	Start	End	Epitope	Percentile Rank	IC50	Method Used
1	H2-IAb	240	254	DGATLNLASSSVKLM	15.00	2054.00	Consensus (smm/nn)
1	H2-IAb	40	54	IIPAIVGGIATGAAV	15.00	2041.00	Consensus (smm/nn)
1	H2-IAd	267	281	AYLAPSYSTINTSKV	15.00	2586.00	Consensus (smm/nn)
1	H2-IAd	155	169	DLDVNMQKATLRLGQ	15.00	2642.00	Consensus (smm/nn)
1	H2-IAd	241	255	GATLNLASSSVKLMG	15.00	3399.00	Consensus (smm/nn)
1	H2-IAd	531	545	NINKLITASTNVAIK	15.00	3596.00	Consensus (smm/nn)
1	H2-IAb	706	720	ATGFYKPLIKINSAQ	15.05	3161.00	Consensus (smm/nn)
1	H2-IAd	295	309	HNAAQAGIIASNKTH	15.05	5323.00	Consensus (smm/nn)
1	H2-IAd	216	230	SSTVLTLQASEGITS	15.05	5366.00	Consensus (smm/nn)
1	H2-IAd	98	112	YDLYKSLLSSKIDGG	15.15	2341.00	Consensus (smm/nn)
1	H2-IAb	289	309	HLTVGDHNAAQAGII	15,20	1284.00	Consensus (smm/nn)
1	H2-IAd	270	284	APSYSTINTSKVTGE	15.25	2049.00	Consensus (smm/nn)
1	H2-IAb	534	548	KLITASTNVAIKNFN	15.50	2571.00	Consensus (smm/nn)
1	H2-IAb	17	31	LALVGALVSITPQQS	15,50	2268.00	Consensus (smm/nn)
1	H2-IAb	31	45	SHAAFFTTVIIPAIV	15.50	1929.00	Consensus (smm/nn)
1	H2-IAd	242	256	ATLNLASSSVKLMGN	15.50	3563.00	Consensus (smm/nn)
1	H2-IAd	532	546	INKLITASTNVAIKN	15.50	3414.00	Consensus (smm/nn)
1	H2-IAd	543	547	NKLITASTNVAIKNF	15.50	3624.00	Consensus (smm/nn)
1	H2-IAb	377	391	TQVINGPFAGGKDTV	15.65	3063.00	Consensus (smm/nn)
1	H2-IAd	405	415	ADGTIRVGGYKASLT	15.75	4843.00	Consensus (smm/nn)
1	H2-IAb	28	42	PQQSHAAFFTTVIIP	16.00	1735.00	Consensus (smm/nn)
1	H2-IAb	378	392	QVINGPFAGGKDTVV	16.00	2421.00	Consensus (smm/nn)
1	H2-IAb	27	41	TPQQSHAAFFTTVII	16.00	1764.00	Consensus (smm/nn)
1	H2-IAd	570	584	DIGSQSRINTVRLET	16.00	2895.00	Consensus (smm/nn)
1	H2-IAd	15	29	VSLALVGALVSITPQ	16.00	3961.00	Consensus (smm/nn)
1	H2-IEd	723	727	IKNTEHVLLKAKIIG	16.00	4815.00	Consensus (smm/nn)

Construction of multi-epitope vaccine

This vaccine was constructed with the 56 CTL and 27 HTL epitopes consisting of amino acid residues and an Adjuvant. The EAAK linker linked CTL epitopes, intra-CTL and Intra-HTL epitopes were linked by AAY and GPGPG linker, respectively. The final vaccine construct has a suitable adjuvant, linker, and immunogenic CTL, and HTL epitopes moving from N-terminal to C-terminal.

Construction of multi-epitope subunit vaccine (Figure 1)

APPHALSEAAYGTATFNNDIAAYKVDAHTANFAAYGIDTGNGGFAAYTTNAAHLHIAAYSKNAEISLYAAYLTTNAAHLHAAYDMKDA
VGTYAAYLLKAKIIGYAAYDIDSATGFYAAYKTEIQPTQVAAYGTATFNNDIAAYKVDAHTANFAAYRSKDIDTLYAAYGIDTGNGGFA
AYTTNAAHLNIAAYDMKDAVGTYAAYATDALNNVAAAYLLKAKIIGYAAYDIDSATGFYAAYKTEIQPTQVAAYSMVNNPDNYAAYK
LVINDFYYAAYSANVEARYYAAYASANVEARYAAYDTNNGTIAFAAYSSDIDTLYAAAYRSSDIDTLYAAYDIDTGNGGFAAYTTNAAH
LIAAYLTTNAAHLHAAYGTSAGVDAYAAYGTDTNNGTIAAYDMKDAVGTYAAYYSAATRASYAAYLLKAKIIGYAAYNVDSATGFYA
AYKTEIQPTQVAAYSMVNNPDNYAAYKLVINDFYYAAYSANVEARYYAAYASANVEARYAAYSSDIDTLYAAAYRSSDIDTLYAAYTS
NARFASYAAYDIDTGNGGFAAYGTSAGVDAYAAYDMKDAVGTYAAYTTNAANLNIAAYLLKAKIIGYAAYTTNAANLNIAAYLLKAKI
IGYAAYNVDSATGFYAAYKTEIQPTQVAAYSMVNNPDNYAAYDMKDAVGTYAAYDMKDAVGTYAAYDMKDAVGTYGPGPGDGATL
NLASSSVKLMGPGPGIIPAIVGGIATGAAVGPGPGAYLAPSYSTINTSKVGPGPHDLDVNMQKATLRLGQGPGPGGATLNLASSSVKLMG
GPGPGNINKLITASTNVAIKGPGPGATGFYKPLIKINSAQGPGPGHNAAQAGIIASNKTHGPGPGSSTVLTLQASEGITSGPGPGYDLYKSLL
SSKIDGGGPGPGHLTVGDHNAAQAGIIGPGPGAPSYSTINTSKVTGEGPGPGKLITASTNVAIKNFNGPGPGLALVGALVSITPQQSGPGPGS
HAAFFTTVIIPAIVGPGPGATLNLASSSVKLMGNGPGPGINKLITASTNVAIKNGPGPGNKLITASTNVAIKNFGPGPGTQVINGPFAGGKDT
VGPGPGADGTIRVGGYKASLTGPGPGPQQSHAAFFTTVIIPGPGPGQVINGPFAGGKDTVVGPGPGTPQQSHAAFFTTVIIGPGPGDIGSQSR
INTVRLETGPGPGVSLALVGALVSITPQGPGPGIKNTEHVLLKAKIIGGPGPGKLITASTNVAVKNFNGPGPGAYLAPSYSTINTSKVGPGPG
DLDVNMQKATLRLGQGPGPGKASLTTNAAHLNIGKGPGPGALVKNAPFAHRATPNGPGPGNAARHYWVKGGQQNKGPGPGATGFYKP
LIKINSAQGPGPGSSTVLTLQASEGITSGPGPGAPSYSTINTSKVTGEGPGPGIPAIVGGIATGTAVGGPGPGIVGGIATGTAVGTVSGPGPGP
AIVGGIATGTAVGTGPGPGSHAAFFTTVIIPAIVGPGPGRPLVSLVLAGALVSAGPGPGEFPNKEYDLYRSLLSGPGPGNVNLEEQFKERLA
LYGPGPGRDLLQTLLIDSHDAGGPGPGIGKAWKNIGISKTANGPGPGLVSLVLAGALVSAIPGPGPGRNAAQAGIIASNKTHGPGPGAIVGG
IATGTAVGTVGPGPGIIPAIVGGIATGTAVGPGPGSNTEVINPPNNTQKTGPGPGDIGSQSRINTVRLETGPGPGTVGDRNAAQAGIIASGPGP
GAARHYWVKGGQQNKLGPGPGIKNTEHVLLKAKIIGGPGPGGVSYNHLGSTNFKSNGPGPGIIPAIVGGIATGAAVGPGPGNTTNLPTNTT
SNARSGPGPGPTQVIDGPFAGAKDTGPGPGQANSLNSGANNTNFGGPGPGQVIDGPFAGAKDTVVGPGPGSITPQKSHAAFFTTVGPGPGS
VGVSYNHLGSTNFKGPGPGAYLAPSYSTINTSKVGPGPGDLDVNMQKATLRLGQGPGPGGDTSYFYMNAGVLQEGPGPGNINKLITASTN
VAIKGPGPGRTMIDATSANEITKQGPGPGTIKVGGYTASLTTNAGPGPGASYGYDFAFFRNALVGPGPGSSTVLTLQASEGITSGPGPGLYQ
FAPKYEKPTNVWGPGPGAAEVLYQFAPKYEKPGPGPGYDLYKSLLSSKIDGGGPGPGISLGRFVNLKASAHTGPGPGALVGALVSITPQKS
HGPGPGKLITASTNVAIKNFNGPGPGNGGNTTNLPTNTTSNGPGPGPSVGVSYNHLGSTNFGPGPGSHAAFFTTVIIPAIVGPGPGSNTQVIN
PPNSGQKTGPGPGINKLITASTNVAIKNGPGPGNKLITASTNVAIKNFGPGPGSITPQKSHAAFFTTVGPGPGFAQRLQALKGQRFASGPGPG
PNKEYDLYKSLLSSKGPGPGNNTNFGVYSRIFANQGPGPGNAPFARYSATPNLVAGPGPGSAAEVLYQFAPKYEKGPGPGYQFAPKYEKP
TNVWAGPGPGIGKAWKNIGISKTANGPGPGNVSAGTNSISNVNLIGPGPGNDFYYAPWNYFDARNGPGPGPSYSTINTSKVAGEVGPGPG
NSRLVNLSRRHTNNIGPGPGAFGPQGSPWGTSNLMGPGPGAKIIGYGNVSAGTNSGPGPGDSNTQVINPPNSGQKGPGPGGFVYLHNLISN
IGHFGPGPGGGFGSYGYSSFNNQAGPGPGGSKISVHYLGNSTPTGPGPGPQKSHAAFFTTVIIPGPGPGVSYNHLGSTNFKSNSGPGPGDIGS
QSRINTVRLETGPGPGTVGDRNAAQAGIIASGPGPGVSLALVGALVSITPQGPGPGALAQNAPFARYSATPGPGPGDAGYARKMIDATSAN
GPGPGGVSYNHLGSTNFESNGPGPGHKTALKNGASSQHLFGPGPGIIPAIVGGIATGAAVGPGPGKLITASTNVAVKNFNGPGPGPTQVIDG
PFAGAKDTGPGPGQVIDGPFAGAKDTVVGPGPGAYLAPSYSTINTSKVGPGPGDLDVNMQKATLRLGQGPGPGGDTSYFYMNAGVLQEG
PGPGIGNQSRINTVRLETGGPGPGTIKVGGYTASLTTNAGPGPGASYGYDFAFFRNALVGPGPGATGFYKPLIKINSAQGPGPGSSTVLTLQ
ASEGITSGPGPGLYQFAPKYEKPTNVWGPGPGAAEVLYQFAPKYEKPGPGPGYDLYKSLLSSKIDGGGPGPGAPSYSTINTSKVTGEGPGP
GISLGRFVNLKASAHTGPGPGLSYGASTRASYGYDFGPGPGATLNVGNAAAMFFNNGPGPGKTALKNGASSQHLFNGPGPGLALVGALV
SITPQQSGPGPGNGGNTTNLPTNTTSNGPGPGNTTNLPTNTTSNARFGPGPGPSVGVSYNHLGSTNFGPGPGSHAAFFTTVIIPAIVGPGPGS
NTQVINPPNSGQKTGPGPGSSDIDTLYAHSGAKGGPGPGSVGVSYNHLGSTNFEGPGPGTNTTSNARFASYAPIGPGPGWANAIGGASLNS
GGNGPGPGQSYNYLSYGASTRASGPGPGSYNYLSYGASTRASYGPGPGPNKEYDLYKSLLSSKGPGPGTNNIDSFAKRLQALKGPGPGNN
ANFGVYSRIFANQGPGPGRDLLQTLLIDSHDAGGPGPGSAAEVLYQFAPKYEKGPGPGYQFAPKYEKPTNVWAGPGPGIGKAWKNIGISK
TANGPGPGNVSAGTNSISNVNLIGPGPGNDFYYAPWNYFDARNGPGPGNSRLVNLSRRHTNNIGPGPGAKIIGYGNVSAGTNSGPGPGAQ
PGATPNLVAINKHGPGPGDSNTQVINPPNSGQKGPGPGGFVYLHNLISNIGHFGPGPGGGFGSYGYSSFNNQAGPGPGGSKISVHYLGNSTP
TGPGPGGSTNFESNSTHKTALGPGPGNSLNSGANNANFGVYGPGPGNTARNPLNTHARVMMGPGPGPQQSHAAFFTTVIIPGPGPGRKMI
DATSANEITKQGPGPGTPQQSHAAFFTTVIIGPGPGTVGDRNAAQAGIIASGPGPGVSLALVGALVSITPQGPGPGYNYLSYGASTRASYGG
PGPGDLDVNMQKATLRLGQGPGPGSSTVLTLQASEGITSGPGPGDLDVNMQKATLRLGQGPGPGSSTVLTLQASEGITSGPGPGDLDVNM
QKATLRLGQGPGPGSSTVLTLQASEGITS

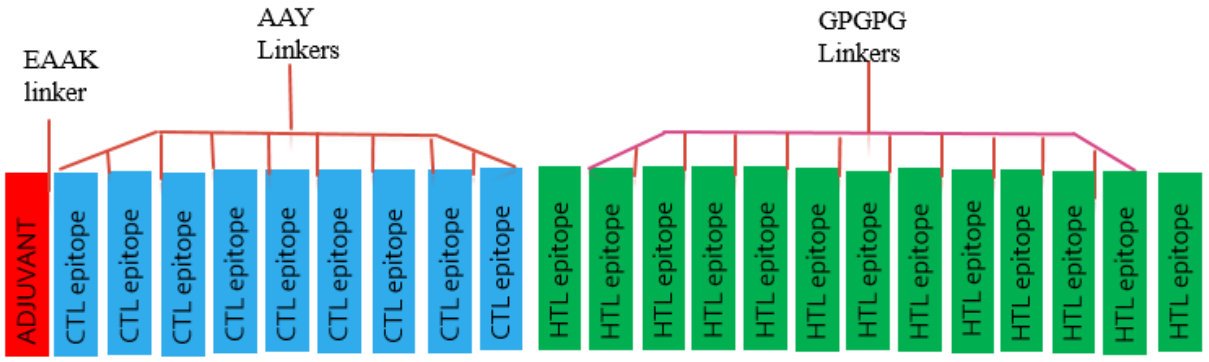

Figure 1: Construction of multi-epitope subunit vaccine

Vaccine allergenicity and antigenicity prediction

AllerTOP online server was used to determine the allergenicity of the predicted vaccine construct; it was found that the vaccine protein is non-allergen in nature and safe for human use. The antigenicity of the designed vaccine construct was determined by the ANTIGENpro server.

Determination of physicochemical properties

The molecular weight (MW) of the final protein was predicted to be 407329.10 Da with the formula: $C_{18146}H_{27908}N_{5008}O_{5636}S_{29}$. The protein is predicted to be alkaline based on the theoretical isoelectric point value (pI) of 9.32. The half-life was assessed to be 4.4 hours in mammalian reticulocytes in vitro, > 20 hours in yeast in vivo and > 10 hours in *Escherichia coli* in vivo. An instability index (II) of 14. Seventy-five classifying the protein as stable. Thermostability was indicated by the estimated aliphatic index predicted to be 67.26, while the predicted Grand average of hydropathicity (GRAVY) was -0.234.

DISCUSSION

Vaccination is one of the utmost applicable processes to proficiently, precipitously, and reasonably advance public health and the preeminent way to constrain infectious disease in society [9]. Up until now, exertions to expound a suitable all-inclusive vaccine against *Helicobacter pylori* bacterial infections have been ineffective. Recent research is centered on subunit vaccines as compared to whole organism vaccines because subunit vaccines contain distinctive immunogenic constituents of the pathogens liable for the infection rather than the whole pathogenic agent [9]. Thus, in this study, the major focus was detecting conserved antigenic epitopes in selected VacA protein sequences.

Different criteria were consecutively exploited to recognize the most suitable epitopes for our vaccine design, and selected T-cell epitopes, which are antigenic and capable of inducing antibody production, were detected. CTL and HTL epitope prediction was carried out base on their need in a vaccine construct to help elicit an effective adaptive immunity for successful protection against pathogen infection [10,11]. However, CTL plays a cardinal role in the battle against infections. Therefore, to a great extent, various studies have elucidated the technique behind its response to microorganisms [12]. Degraded proteins of microorganisms found on the surface of infected cells are presented to the CTLs in combination with the MHC Class I molecules. Upon recognizing the degraded part of such proteins by CTLs, cytotoxic granules are released, thus leading to the death of the infected cells [12]. Filtering through various parameters, 56 CTL epitopes have been extracted in this study for the construction of the candidate vaccine.

Following the cleavage of specific antigens to body cells, antigen-presenting cells readily present the epitopes to HTLs in the form of epitope-MHC class II complex. This interaction leads to the activation of HTLs, which thereby result in the secretion of a wide range of cytokines and chemokines such as IFNγ, IL-4, IL-10 etc. that play diverse roles in the immune response against invaders upon recognition [12]. Based on the analysis from this study, 94 HTL epitope was selected for the vaccine construction. The detected epitopes entail experimental validation for their possible use in peptide vaccines. Since immune informatics is a field that can predict a priori whether an antigen is immunogenic through epitope prediction, our focus was to stage in silico prediction of T-cell epitopes within the amino acid sequence of VacA for the design of a subunit vaccine against *H. pylori*. A passable humoral response is a contingent upon an appropriate CD4+ T-cell response.

Furthermore, vaccines that induce cellular immune responses are crucial for infections initiated by intracellular pathogens. One of the main drawbacks in developing such vaccines includes obscurity in recognizing relevant T-cell antigens that stimulate protective cellular immunity [10]. T-cells detect the peptide epitopes posed by the MHC molecules, which are the surface proteins of antigen-presenting cells recognized by the T-cell receptors (TCR). These antigen-presenting molecules (MHC) are either class I or II. MHC class I molecules pose on nearly all nucleated cells that specifically signify the endogenous proteins or antigen processed through the cytosolic pathway and denotes cytotoxic T lymphocytes (CTLs). MHC class II molecules pose exogenous antigens, ordinarily, surface proteins of the pathogens, which are processed through endocytic pathways and accessible to helper T lymphocytes or CD4+ T cells [9]. In this study, novel immune-informatics tools were exploited to design a prospective, benign and immunogenic subunit vaccine that may have the proficiency to constrain *Helicobacter pylori* infections.

CONCLUSION

VacA has probable B-cell, and T-cell epitopes dispersed all over the whole sequence. Various fragments could be designated as candidates for the design of a vaccine against *Helicobacter pylori*. Consequently, in this study, seven sequences comprising both B-cell and T-cell epitopes were distinguished that may be used to spawn both humoral and cellular responses. These antigenic peptides can be significant candidates for indicative, curative, and preventive vaccines against *H. pylori.* Selected fragments of VacA that contains B- and T-cell epitopes in conjunction with effective adjuvants and/or delivery systems may facilitate the enhancement of an effective *H. pylori* vaccine that prompts a Th1-mediated and humoral protective immune response that does not instigate antagonistic immune-pathologies. The results of our study provide analytical data for the detection and assessment of epitopes in VacA and may be exploited for the development of epitope vaccines that have improved safety and efficacy.

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