Epitope-Based Peptide Vaccine Design Against Fructose Bisphosphate Aldolase of *Candida Glabrata*: An Immunomics Approach

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Abstract

Background: *Candida glabrata* is a human opportunistic pathogen that can cause life-threatening systemic infections. Although, there are multiple effective vaccines against fungal infections, and some of these vaccines were engaged in different stages of clinical trials, none of them yet approved by (FDA).

Aim: To predict the most conserved and immunogenic B- and T-cell epitopes from the Fructose Bisphosphate aldolase (Fba1) protein of *C. glabrata*.

Materials and Methods: 13 *C. glabrata* Fructose bisphosphate aldolase protein sequences (361 amino acid) were retrieved from NCBI and several in silico tools presented in the IEDB server for predicting peptides were used and homology modeling and molecular docking were performed.

Result: The promising B-cell Epitopes were AYFKPH, VDKESELYTK, and HVDKESELYTK. While, promising peptides which have the high affinity to MHC I binding were: AVHEALAPI, KYFKRMAAM, QTSNGGAAY, RMAAMNQWL and YFKEHGEPL. Two peptides (LFSSHMLDL and YIRSIAPAY) were noted to have the highest affinity to MHC class II that interact with 9 MHC class II alleles. The molecular Docking revealed the epitopes QTSNGGAAY and LFSSHMLDL have the high binding energy to MHC molecules

Conclusion: The epitope-based vaccines predicted by using immunoinformatics tools have remarkable advantages over the conventional vaccines that they are more specific, less time consuming, safe, less allergic and more antigenic. Further in vivo and in vitro experiments are needed to prove the effectiveness of the best candidates epitopes (QTSNGGAAY and LFSSHMLDL). To the best of our knowledge, this is the first study that has predicted B- and T- cells epitopes from Fba1 protein by using in silico tools in order to design an effective epitope-based vaccine against *C. galabrata*.

Key words: *Candida glabrata*, Fructose Bisphosphate aldolase, Peptide Vaccine.

1. Introduction

Candidiasis is a fungal infection that has a high burden of morbidity and mortality in hospitalized and immunocompromised patients. It occurs in more than a quarter of a million patients every year with incidence rates for candidemia of 2–14 per 100000 [1-4]. *Candida* pathogenicity is facilitated by a number of virulence factors, most importantly adherence to host surfaces including medical devices, biofilm formation and secretion of hydrolytic enzymes. Also, *Candida* cells elaborate polysaccharides, proteases, phospholipases and haemolysins that cause host cell damage which leads to the increase in the incidence and antifungal resistance of NCAC species, specifically *C. glabrata* and the unacceptably high morbidity and mortality associated with these species^[5].

Candida glabrata (*C. glabrata*) is a human opportunistic pathogen that can cause life-threatening systemic infections. *C. glabrata* is not polymorphic, grow as blastoconidia (yeast), lack of pseudohyphal formation so classified in the genus *Torulopsis* *C. glabrata* cells (1–4 µm in size)^[6] and forms glistening, smooth, and cream-colored colonies^[7]. During infection, invade the innate immune system such as macrophages, which belong to the first line of defense against invading pathogens. *C. glabrata* able to modify its phagosomal compartment, avoiding full maturation, acidification and thus prevent the reaching of not the hostile phagolysosomal environment ^[8]. *C. glabrata* is able to invade the bloodstream and different organs in a mouse model that has intragastrintestinal infections^[9]. The genome of *C. glabrata* was published in 2004 by Dujon *et al.* 2004^[10]. *C. glabrata* has a haploid genome that allows adaptation to a wide range of environments [5, 11, 12]. Also, its genome contains more tandem repeats of genes than the other *Nakaseomyces*^[13] *C. glabrata* covers 67 genes encoding putative adhesin which is cell wall proteins, including the Epa family with 17 members^[11, 14]. Most importantly examples are Epithelial Adhesin 1 (Epa1p)^[15] and Fructose bisphosphate aldolase protein (Fba1) which play an essential role in the pathogenicity of *Candida* species mainly in the adhesion of the pathogen to the host ^[16, 17].

Fba1 is a yeast cell wall protein that presents in multiple species of *Candida*^[18]. e.g *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. albicans* fungal pathogen.^[19-22] It is a very important enzyme in the glycolytic pathway^[23-26] and multifunctional protein^[27] that can

facilitate the attachment (adhesion) to human cells or abiotic surfaces^[28-30], protects *Candida* cells from the host's immune system^[28] and promotes the detoxification of the ROS generated during the respiratory burst^[28, 29, 31]. However, proteomics analysis revealed that Fba1 is the most abundant and stable enzyme in *Candida*. Moreover, it is considered one of the main immunodominant proteins^[32, 33] in *Candida* cells and tested in the murine model as protected protein against candida^[34] especially, *C. albicans*; also introduced immunity to *C. glabrata*^[35] therefore Fba1 is a potential antifungal target in yeast^[36]. Multiple vaccines used Fba1 as immunogenic protein against different pathogens such as the lethal challenging *S. pneumonia*, *salmonella spp.*, and *M. bovis*^[37-39].

The incidence of fungal infection has been increasing in the latest years, due to several factors such as misuse of broad-spectrum antibiotics, cytotoxic chemotherapy, immunocompromised patients, and transplantations^[40, 41]. Invasive fungal infections are a major cause of global morbidity and mortality, accounting for about 1.4 million deaths per year^[42]. Systemic fungal infections cost the healthcare industry approximately \$2.6 billion per year in the USA alone^[43]. However, *Candida* species pose a base problem in hospitals, according to Healthcare-Associated Infections (HAI)^[44-47]. Although, there are multiple effective vaccines against fungal infections, and some of these vaccines were engaged in different stages of clinical trials, none of them yet approved by (FDA)^[48]. Therefore, there is an urgent and crucial need to design vaccines against *candida* species that might improve the quality of life for immunosuppressed patients^[49]. The aim of this study is to predict the most conserved and immunogenic B- and T-cell epitopes from the Fba1 protein of *C. glabrata* by using in silico tools presented in the IEDB server. The Immunoinformatics approach has multiple benefits in comparison to other approaches by being affordability, safety, time-saving and clinically applicable using different computational software techniques^[50-52]. To the best of our knowledge, this is the first study that has predicted the best candidates of multiple epitopes of Fba1 protein against *C. glabrata*.

2. Materials and Methods

Figure 1. Schematic representation of the methodology phases

2.1 Retrieving of Fructose Bisphosphate Aldolase protein sequences:

13 *Candida glabrata* Fructose bisphosphate aldolase protein sequences (361aa proteins) were retrieved from NCBI (<https://www.ncbi.nlm.nih.gov/protein>) Database on 21 January 2019 ^[17].

2.2 Determination of conserved regions

Multiple sequence alignment (MSA) was used to determine the conserved regions, the retrieved sequences were aligned by MSA using Clustal-W as Applied in the Bio-Edit [53].

2.3 Prediction of B-cell epitope:

The reference sequence of Fructose biphosphate aldolase protein was submitted to the following different B cell tests [54].

2.3.1 Prediction of linear B-cell epitopes:

Bepipred tool from IEDB (<http://tools.iedb.org/bcell/result/>) was used to predict the linear B-cell epitopes from the conserved region with a default threshold value of 0.350 [55-57].

2.3.2 Prediction of surface accessibility:

Emini surface accessibility prediction tool of the immune epitope database (IEDB) (<http://tools.iedb.org/bcell/result/>) was used to predict the surface epitopes from the conserved region with the default threshold value 1.0 [58]

2.3.3 Prediction of epitopes antigenicity:

Kolaskar and Tongaonkar antigenicity method was used to detect the antigenic sites with a default threshold value of 1.025, (<http://tools.iedb.org/bcell/result/>) [59].

2.3.4 Prediction of discontinuous B-cell Epitope:

The modeled 3D structure was submitted to ElliPro prediction tool to filter out the antigenic residues. The minimum score and maximum distance (Angstrom) were calibrated in the default mode with a score of 0.5, and 6, respectively [60].

2.3 Prediction of MHC class I binding epitopes:

The peptide binding affinity to MHC1 molecules was defined by the IEDB MHC I prediction tool at <http://tools.iedb.org/mhc1>. The binding affinity of Fructose biphosphate aldolase peptide to MHC1 molecules was obtained using artificial neural network (ANN) method. All conserved epitopes that bind to MHC1 alleles at score ≤ 500 half-maximal inhibitory concentrations (IC₅₀) with 9 peptides length were selected for further analysis [61-66].

2.5 MHC class II binding predictions:

Prediction of peptide binding affinity to MHCII molecules was defined by the IEDB MHC II prediction tool at (<http://tools.iedb.org/mhcii/result/>). MHC II molecule has the ability to bind different lengths Peptides which makes prediction accuracy debatable. For MHC II binding predication, human allele references set were used.

The prediction method was selected as NN-align to asses both the binding affinity and MHC II binding core epitopes with 9 peptides length at score IC50 of 100 ^[67].

2.6 Population coverage calculation:

The candidates epitopes of MHC I and MHC II and combined binding of MHCI and MHC II alleles from *Candida glabrata* Fructose biphosphate aldolase protein were employed for population coverage, and the world population was set as a target population for the selected MHC I and MHC II combined binding alleles using IEDB population coverage calculation tool at <http://tools.iedb.org/population/> ^[68].

2.7 Homology modeling:

The reference sequence of *Candida glabrata* Fructose biphosphate aldolase protein was applied to Raptor X for modeling at: (<http://raptorx.uchicago.edu/>). Then the 3D structural model of the protein was visualized by using Chimera a tool powered by UCSF. ^[69-73].

2.8 The Physicochemical Properties:

The function of vaccines is to enhance the immunogenic response once introduced to the immune system. Thus it is essential to recognize the physiochemical parameters of the protein protogram and Bio edit were implemented to assess the Physicochemical Properties. ^[53] (Available at: <https://web.expasy.org/protparam/>, and <https://web.expasy.org/protscale/>) ^[74].

2.9 Molecular docking analysis

Molecular docking was performed using Moe 2007. The 3D structures of the promiscuous epitopes were predicted by PEP-FOLD. The crystal structure of HLA A*02:06 (PDB ID 3OXR) and HLA-DRB1*01:01(PDB ID 5JLZ) were chosen as a model for molecular docking and were downloaded in a PDB format from the RCSB PDB resource. However, the selected crystal structures were in a complex form with ligands. Thus, to simplify the complex structure all water molecules, hetero groups and ligands were removed by Discovery Studio Visualizer 2.5. Partial charge and energy

minimization were applied for ligands and targets. In terms of the identification of the binding groove, the potential binding sites in the crystal structure were recognized using the Alpha Site Finder. Finally, ten independent docking runs were carried out for each Peptide. The results were retrieved as binding energies. Best poses for each epitope that displayed lowest binding energies were visualized using UCSF chimera 1.13.1 software [72, 75-78].

3. Result

3.1.1 B-cell epitope prediction:

The reference sequence of Fructose biphosphate aldolase from *C. glabrata* was analyzed using Bepipred Linear Epitope Prediction first, the average binder's score of the protein to B cell was 0.199, minimum was -0.009 and 2.424 for a maximum score, all values equal or greater than the default threshold 0.350 were potentially linear epitopes (Table 1 and figure 1).

Table 1: Predicted antigenic B-cell epitopes, 9 antigenic sites were identified from Fructose biphosphate aldolase of *C. glabrata*.

Start	End	Peptide	Length
63	70	SNGGAAYF	8
73	84	KGVSNDGQNASI	12
129	134	AYFKEH	6
147	155	SEETDDENI	9
178	199	ITGGEEDGVNNEHVDKESLYTK	22
247	260	KYAAEKTGAPAGSK	14
269	280	GSGSTQEEFNTG	12
318	331	GNPEGADKPNKKFF	14
336	345	WVREGEKTMS	10

In Emini surface accessibility prediction, for a potent B-cell epitope the average surface accessibility area of the protein was scored as 1.000, with a maximum of 7.725 and a minimum of 0.113, all values equal or greater than the default threshold 1.000 were potentially in the surface. In addition, Kolaskar and Tongaonkar antigenicity prediction's

average of antigenicity was 1.025, with a maximum of 1.223 and minimum of 0.853; all values equal to or greater than the default threshold 1.025 are potential antigenic determinants. The results of all conserved predicted B cell epitopes are shown in (table 2 and figure 2).

Table 2. List of the most promising B- cell Epitope and their surface and Antigenicity (c=conserve, p=pathogenicity).

Start	End	Peptide	Length	Conserve	Surface	Surface Score (Emini's surface threshold =1.000)	Antigenicity	Antigenicity Score(Kolaskar's Test =1.025)
129	134	AYFKEH	6	C	P	1.502	P	1.034
191	199	VDKESLYTK	9	C	P	2.48	P	1.032
190	199	HVDKESLYTK	10	C	P	2.648	P	1.04

Figure2: Bepipred Linear Epitope Prediction, The Red Line is the threshold, above (the yellow part) is proposed to be part of the B-cell Epitope.

Figure 3: Emini's Surface Accessibility Prediction Test, The Red Line is the threshold above (the yellow part) is proposed to be part of the B-cell Epitope.

Figure 4: Kolaskar and Tongaonkar antigenicity prediction Test, the Red Line is the threshold, above (the yellow part) is proposed to be part of the B-cell Epitope.

3.1.2. Discontinuous B-cell Epitope Prediction:

The modeled 3D structure was submitted to ElliPro prediction tool to filter out the antigenic residues. The minimum score and maximum distance (Angstrom) were calibrated in the default mode with a score of 0.5, and 6, respectively (Table3 and Figure 4).

Table 3. List of four predicted non-linear B-cell epitopes with highest number of Residues and their scores using ElliPro tool.

No.	Residues	Number of residues	Score	3D structure
1	_:T300, _:G301, _:I302, _:R303, _:D304, _:Y305, _:V306, _:L307, _:N308, _:K309, _:K310, _:D311, _:Y312, _:I313, _:M314, _:S315, _:M316, _:V317, _:G318, _:N319, _:P320, _:E321, _:G322, _:A323, _:D324, _:K325, _:P326, _:N327, _:K328, _:K329, _:F330, _:F331, _:E339, _:K342	34	0.867	

2	_:D332, _:P333, _:R334, _:V335, _:W336	5	0.749
3	_:V3, _:Q4, _:E5, _:V6, _:L7, _:K8, _:Y25, _:E28, _:H29, _:K30, _:F31, _:K55, _:S56, _:A156, _:T157, _:V159, _:K160, _:K163, _:G177, _:I178, _:T179, _:G180, _:G181, _:E182, _:E183, _:D184, _:G185, _:V186, _:N187, _:N188, _:E189, _:H190, _:V191, _:D192, _:K193, _:E194, _:S195, _:L196, _:Y197, _:T198, _:K199, _:P200, _:E201, _:F204, _:A205, _:E208, _:A209, _:A211, _:P212, _:I213, _:S214, _:P215, _:A222, _:F223, _:G224, _:Q231, _:A232, _:G233, _:N234, _:V235, _:V236, _:L237, _:S238, _:P239, _:E240, _:A243, _:D244, _:K247, _:Y248, _:A249, _:A250, _:E251, _:K252, _:T253, _:G254, _:A255, _:P256, _:A257, _:G258, _:S259, _:K260, _:P261, _:S272, _:T273, _:Q274, _:E275, _:N278, _:T279, _:N282, _:N283, _:T357, _:K358, _:N359, _:T360, _:L361	95	0.669
4	_:V15, _:G16, _:A71, _:G72, _:K73, _:G74, _:V75, _:S76, _:N77, _:D78, _:G79, _:Q80, _:N81, _:A82, _:I84, _:R85, _:C112, _:A113, _:K114, _:L117, _:P118, _:D121, _:G122, _:L124, _:E125, _:A126, _:E128, _:A129, _:Y130, _:F131, _:K132, _:E133, _:H134, _:G135, _:E136, _:P137, _:L138, _:R164, _:A166, _:A167, _:M168, _:N169, _:Q170	43	0.668
5	_:L146, _:S147, _:E148, _:E149, _:T150, _:D151, _:D152, _:E153	8	0.582
6	_:R9, _:K10, _:T11, _:G12, _:I14, _:R52, _:D53, _:A98, _:P99, _:A100, _:Y101, _:G102, _:I103	13	0.514

Figure 5: Structural position of the promising B-cell Epitope (AYFKPH, VDKESELYTK, and HVDKESELYTK).

3.2 T-cell peptide prediction:

3.2.1. Prediction of MHC-I binding profile for T cytotoxic cell conserved epitopes:

114 epitopes were anticipated to interact with different MHC-1 alleles. The core epitopes (KYFKRMAAM and QTSNGGAAY) were noticed to be the dominant binders with 7

alleles for each (HLA-A*24:02, HLA-A*30:01, HLA-A*31:01, HLA-B*14:02, HLA-C*07:02, HLA-C*12:03, HLA-C*14:02) (HLA-A*01:01, HLA-A*26:01, HLA-A*29:02, HLA-A*30:02, HLA-B*15:01, HLA-B*15:02, HLA-B*35:01) followed by AVHEALAPI, RMAAMNQWL, and YFKEHGEPL which binds with five alleles (table IV).

Table IV: List of most promising epitopes that had a good binding affinity with MHC-I alleles in terms of IC50 and Percentile rank.

Peptide	Start	End	Allele	ic50
KYFKRMAAM	160	168	HLA-A*24:02	451.84
	160	168	HLA-A*30:01	232.12
	160	168	HLA-A*31:01	131.22
	160	168	HLA-B*14:02	427.02
	160	168	HLA-C*07:02	149.13
	160	168	HLA-C*12:03	240.46
	160	168	HLA-C*14:02	6.27
AVHEALAPI	205	213	HLA-A*02:01	154.37
	205	213	HLA-A*02:06	9.78
	205	213	HLA-A*30:01	20.96
	205	213	HLA-A*32:01	122.32
	205	213	HLA-A*68:02	55.22
RMAAMNQWL	164	172	HLA-A*02:01	52.44
	164	172	HLA-A*02:06	237.09
	164	172	HLA-A*32:01	79.39
	164	172	HLA-B*15:01	258
	164	172	HLA-C*14:02	482
QTSNGGAAY	61	69	HLA-A*01:01	54.18
	61	69	HLA-A*26:01	89.37
	61	69	HLA-A*29:02	56.68
	61	69	HLA-A*30:02	47.89
	61	69	HLA-B*15:01	111.57
	61	69	HLA-B*15:02	82.52
	61	69	HLA-B*35:01	99.45
YFKEHGEPL	130	138	HLA-B*08:01	295.97
	130	138	HLA-C*03:03	42.03
	130	138	HLA-C*07:02	319.29
	130	138	HLA-C*12:03	26.8
	130	138	HLA-C*14:02	18.47

3.2.2 Prediction of MHC-II binding profile for T helper cell conserved epitopes:

102 conserved predicted epitopes were found to interact with MHC-II alleles. The core epitopes (LFSSHMLDL) is thought to be the top binder as it interacts with 9 alleles; (HLA-DRB1*07:01, HLA-DPA1*01, HLA-DPB1*04:01, HLA-DPA1*01:03, HLA-DPB1*02:01, HLA-DPA1*02:01, HLA-DPB1*01:01, HLA-DPA1*03:01, and HLA-DPB1*04:02) Followed by IRGSIAAAH which binds to five alleles and VVALEAAR also bind with five alleles but low frequent. Followed by YQAGNVVLS, and IAPAYGIPV (Table V).

Table V: Promising T cell epitope (class MHC II Alleles) with their position and peptide sequence and ic50 value and rank

Core Sequence	Allele	Start	End	Peptide Sequence	IC50	Rank
LFSSHMLDL	HLA-DRB1*07:01	132	146	KEHGEPLFSSHMLDL	17.8	3.37
	HLA-DRB1*07:01	133	147	EHGEPLFSSHMLDLS	24.9	4.81
	HLA-DRB1*07:01	134	148	HGEPLFSSHMLDLSE	40.1	7.21
	HLA-DRB1*07:01	135	149	GEPLFSSHMLDLSEE	69.7	11.12
	HLA-DRB1*07:01	136	150	EPLFSSHMLDLSEET	92.8	13.47
	HLA-DPA1*01/DPB1*04:01	135	149	GEPLFSSHMLDLSEE	93.6	5.05
	HLA-DPA1*01/DPB1*04:01	136	150	EPLFSSHMLDLSEET	95	5.1
	HLA-DPA1*01/DPB1*04:01	134	148	HGEPLFSSHMLDLSE	97.8	5.21
	HLA-DPA1*01:03/DPB1*02:01	133	147	EHGEPLFSSHMLDLS	46	4.82
	HLA-DPA1*01:03/DPB1*02:01	134	148	HGEPLFSSHMLDLSE	48.2	4.99
	HLA-DPA1*01:03/DPB1*02:01	132	146	KEHGEPLFSSHMLDL	50.3	5.16
	HLA-DPA1*01:03/DPB1*02:01	135	149	GEPLFSSHMLDLSEE	53.4	5.4
	HLA-DPA1*01:03/DPB1*02:01	136	150	EPLFSSHMLDLSEET	59.3	5.82
	HLA-DPA1*02:01/DPB1*01:01	134	148	HGEPLFSSHMLDLSE	59	6.3
	HLA-DPA1*02:01/DPB1*01:01	135	149	GEPLFSSHMLDLSEE	59.3	6.33
	HLA-DPA1*02:01/DPB1*01:01	136	150	EPLFSSHMLDLSEET	61.2	6.54
	HLA-DPA1*02:01/DPB1*01:01	133	147	EHGEPLFSSHMLDLS	61.9	6.62
	HLA-DPA1*02:01/DPB1*01:01	132	146	KEHGEPLFSSHMLDL	71.7	7.69
	HLA-DPA1*03:01/DPB1*04:02	135	149	GEPLFSSHMLDLSEE	12	1.14
	HLA-DPA1*03:01/DPB1*04:02	136	150	EPLFSSHMLDLSEET	12.5	1.21
	HLA-DPA1*03:01/DPB1*04:02	134	148	HGEPLFSSHMLDLSE	13.4	1.35
	HLA-DPA1*03:01/DPB1*04:02	133	147	EHGEPLFSSHMLDLS	17.8	1.99
	HLA-DPA1*03:01/DPB1*04:02	137	151	PLFSSHMLDLSEETD	19.7	2.25
	HLA-DPA1*03:01/DPB1*04:02	132	146	KEHGEPLFSSHMLDL	30	3.58
	HLA-DPA1*03:01/DPB1*04:02	138	152	LFSSHMLDLSEETDD	31	3.7
IRGSIAAAH	HLA-DRB1*01:01	81	95	NASIRGSIAAAHYIR	31.5	15.98
	HLA-DRB1*01:01	82	96	ASIRGSIAAAHYIRS	44.5	19.86
	HLA-DRB1*04:01	81	95	NASIRGSIAAAHYIR	86.5	7.02
	HLA-DRB5*01:01	81	95	NASIRGSIAAAHYIR	7.3	1.55
	HLA-DRB5*01:01	82	96	ASIRGSIAAAHYIRS	10.1	2.4
	HLA-DRB5*01:01	80	94	QNASIRGSIAAAHYI	12.7	3.11

	HLA-DRB5*01:01	83	97	SIRGSIAAAHYIRSI	16	3.96
	HLA-DRB5*01:01	79	93	GQNASIRGSIAAAHY	21.7	5.25
	HLA-DRB5*01:01	84	98	IRGSIAAAHYIRSI	26.5	6.23
	HLA-DRB5*01:01	78	92	DGQNASIRGSIAAAH	42.1	8.88
	HLA-DQA1*01:02/DQB1*06:02	80	94	QNASIRGSIAAAHYI	59.3	3.74
	HLA-DQA1*01:02/DQB1*06:02	79	93	GQNASIRGSIAAAHY	62.1	3.98
	HLA-DQA1*01:02/DQB1*06:02	78	92	DGQNASIRGSIAAAH	70.1	4.67
	HLA-DQA1*01:02/DQB1*06:02	81	95	NASIRGSIAAAHYIR	84.4	5.86
	HLA-DQA1*05:01/DQB1*03:01	81	95	NASIRGSIAAAHYIR	4.6	0.27
	HLA-DQA1*05:01/DQB1*03:01	80	94	QNASIRGSIAAAHYI	4.7	0.29
	HLA-DQA1*05:01/DQB1*03:01	79	93	GQNASIRGSIAAAHY	5	0.34
	HLA-DQA1*05:01/DQB1*03:01	82	96	ASIRGSIAAAHYIRS	5.1	0.36
	HLA-DQA1*05:01/DQB1*03:01	83	97	SIRGSIAAAHYIRSI	5.2	0.37
	HLA-DQA1*05:01/DQB1*03:01	78	92	DGQNASIRGSIAAAH	5.4	0.41
	HLA-DQA1*05:01/DQB1*03:01	84	98	IRGSIAAAHYIRSI	5.7	0.48
YQAGNVVLS	HLA-DRB1*01:01	227	241	HGVYQAGNVVLSPEI	19.7	11.15
	HLA-DRB1*01:01	228	242	GVYQAGNVVLSPEIL	29.5	15.29
	HLA-DRB1*09:01	227	241	HGVYQAGNVVLSPEI	80.9	5.58
	HLA-DRB1*09:01	228	242	GVYQAGNVVLSPEIL	96.9	6.69
	HLA-DQA1*01:02/DQB1*06:02	227	241	HGVYQAGNVVLSPEI	91.3	6.42
	HLA-DQA1*05:01/DQB1*03:01	224	238	GNVHGVYQAGNVVLS	7.9	0.96
	HLA-DQA1*05:01/DQB1*03:01	226	240	VHGVYQAGNVVLSPE	7.9	0.96
	HLA-DQA1*05:01/DQB1*03:01	225	239	NVHGVYQAGNVVLSPE	8.2	1.03
	HLA-DQA1*05:01/DQB1*03:01	227	241	HGVYQAGNVVLSPEI	8.7	1.14
	HLA-DQA1*05:01/DQB1*03:01	228	242	GVYQAGNVVLSPEIL	11	1.67
	HLA-DQA1*05:01/DQB1*03:01	229	243	VYQAGNVVLSPEILA	25.7	4.88
	HLA-DQA1*05:01/DQB1*03:01	230	244	YQAGNVVLSPEILAD	29.8	5.67
VVAALEAAR	HLA-DRB1*03:01	41	55	SSTVVAALEAARDAK	50.9	2.91
	HLA-DRB1*03:01	40	54	SSSTVVAALEAARDA	98.4	4.96
	HLA-DRB1*09:01	41	55	SSTVVAALEAARDAK	95.1	6.59
	HLA-DRB5*01:01	41	55	SSTVVAALEAARDAK	15	3.71
	HLA-DRB5*01:01	42	56	STVVAALEAARDAKS	20.8	5.06
	HLA-DRB5*01:01	40	54	SSSTVVAALEAARDA	28.2	6.54
	HLA-DRB5*01:01	43	57	TVVAALEAARDAKSP	34.8	7.72
	HLA-DRB5*01:01	39	53	TSSSTVVAALEAARD	52	10.26
	HLA-DRB5*01:01	44	58	VVAALEAARDAKSPI	65.6	11.92
	HLA-DRB5*01:01	38	52	VTSSSTVVAALEAAR	73.4	12.78
	HLA-DQA1*01:02/DQB1*06:02	40	54	SSSTVVAALEAARDA	38.1	1.93
	HLA-DQA1*01:02/DQB1*06:02	41	55	SSTVVAALEAARDAK	42.4	2.29
	HLA-DQA1*01:02/DQB1*06:02	42	56	STVVAALEAARDAKS	52.5	3.16
	HLA-DQA1*01:02/DQB1*06:02	43	57	TVVAALEAARDAKSP	85.3	5.93
	HLA-DQA1*05:01/DQB1*03:01	42	56	STVVAALEAARDAKS	16.2	2.87
	HLA-DQA1*05:01/DQB1*03:01	43	57	TVVAALEAARDAKSP	17	3.05
	HLA-DQA1*05:01/DQB1*03:01	41	55	SSTVVAALEAARDAK	17.2	3.09
	HLA-DQA1*05:01/DQB1*03:01	40	54	SSSTVVAALEAARDA	19.5	3.59
	HLA-DQA1*05:01/DQB1*03:01	44	58	VVAALEAARDAKSPI	23	4.35
	HLA-DQA1*05:01/DQB1*03:01	39	53	TSSSTVVAALEAARD	23.7	4.49
	HLA-DQA1*05:01/DQB1*03:01	38	52	VTSSSTVVAALEAAR	30.8	5.85
IAPAYGIPV	HLA-DRB1*01:01	94	108	IRSIAPAYGIPVVLH	12.1	6.74

HLA-DRB1*01:01	93	107	YIRSIAPAYGIPVVL	14.2	8.11
HLA-DRB1*01:01	95	109	RSIAPAYGIPVVLHS	21.8	12.15
HLA-DRB1*01:01	96	110	SIAPAYGIPVVLHSD	45.3	20.07
HLA-DRB1*01:01	97	111	IAPAYGIPVVLHSDH	82.3	27.53
HLA-DRB1*07:01	91	105	AHYIRSIAPAYGIPV	34.1	6.37
HLA-DRB1*07:01	92	106	HYIRSIAPAYGIPVV	39.7	7.15
HLA-DRB1*07:01	93	107	YIRSIAPAYGIPVVL	50.5	8.77
HLA-DRB1*15:01	94	108	IRSIAPAYGIPVVLH	79.2	8.07
HLA-DQA1*05:01/DQB1*03:01	94	108	IRSIAPAYGIPVVLH	16.5	2.94
HLA-DQA1*05:01/DQB1*03:01	96	110	SIAPAYGIPVVLHSD	22.9	4.33
HLA-DQA1*05:01/DQB1*03:01	95	109	RSIAPAYGIPVVLHS	23.7	4.49
HLA-DQA1*05:01/DQB1*03:01	97	111	IAPAYGIPVVLHSDH	30.9	5.86

3.3 Population coverage:

The most interesting findings in this test is that the population coverage analysis result for most common binders to MHC-I and MHC-II alleles per each and combined; exhibit an exceptional coverage with percentages 92.54 %, 99.58 and 98,5 % Respectively.

3.3.1 Population coverage for isolated MHC-I:

Five epitopes are given to interact with the most frequent MHC class I alleles (**AVHEALAPI**, **KYFKRMAAM**, **QTSNGGAAY**, **RMAAMNQWL** and **YFKEHGEPL**), representing a considerable coverage against the whole world population. The maximum population coverage percentage over these epitopes is 92.54% (figure 5).

Figure 6. Illustrates the global coverage for the top five MHC-I peptides (**AVHEALAPI**, **KYFKRMAAM**, **QTSNGGAAY**, **RMAAMNQWL** and **YFKEHGEPL**) Note: In the graph, the line (-o-) represents the cumulative percentage of population coverage of the epitopes; the bars represent the population coverage for each epitope.

3.3.2 Population coverage for isolated MHC-II:

Three epitopes were assumed to interact with the most frequent MHC class II alleles (IRGSIAAAH, LFSSHMLDL and VVAALEAAR) with percentage of 99.58%. The **LFSSHMLDL** epitope show an exceptional result for population coverage test for MHC II binding affinity of 96.60 % globally (figure 6).

Figure 7. Illustrates the global proportion for the top five MHC-II IRGSIAAAH, LFSSHMLDL and VVAALEAAR. Notes: In the graph, the line (-o-) represents the cumulative percentage of population coverage of the epitopes; the bars represent the population coverage for each epitope.

3.3.3 Population coverage for MHC-I and MHC-II alleles combined:

Regarding MHC-I and MHC-II alleles combined, five epitopes were supposed to interact with most predominant MHC class I and MHC class II alleles (IAPAYGIPV, AAFGNVHGV, VVAALEAAR, YIRSTIAPAY and YQAGMVVLS) represent a significant global coverage by IEDB population coverage tool. Reveal coverage with percentage of 98.50 % (figure 7).

Figure 8: Illustrates the global population proportion for the top five MHC-I & II epitopes in combined mode (IAPAYGIPV, AAFGNVHGV, VVAALEAAR, YIRSTIAPAY and YQAGMVVLS). Notes: In the graphs, the line (-o-) represents the cumulative percentage of population coverage of the epitopes; the bars represent the population coverage for each epitope.

3.4 Physiochemical properties :

Predicted as 1 domain(s). Best template: 1dosA, p-value 4.00e-09. Overall uGDT (GDT): 299 (82). 361(100%) residues are modeled. 36(9%) positions predicted as disordered. Secondary struct: 44%H, 10%E, And 44% C. Solvent access: 34%E, 27%M, and 38% B.

Table VI: Amino acids composition of the protein (Fructose-bisphosphate aldolase) with their number and molecular weight (Mol %) using Bio edit software version 7.0.5.3.

Amino Acid	Number	Mol%	Amino Acid	Number	Mol%
Ala A	39	10.80	Leu L	23	6.37
Cys C	3	0.83	Met M	9	2.49
Asp D	21	5.82	Asn N	19	5.26
Glu E	26	7.20	Pro P	15	4.16
Phe F	14	3.88	Gln Q	8	2.22
Gly G	30	8.31	Arg R	10	2.77
His H	12	3.32	Ser S	23	6.37
Ile I	20	5.54	Thr T	17	4.71
Lys K	25	6.93	Val V	29	8.03
Trp W	3	0.83	Tyr Y	15	4.16

Figure 9: Graph showing amino acid composition of Fructose bisphosphate aldolase protein and their molecular weight using Bio edit software 7.0.5.3

3.5 Molecular docking:

Table VII. Docking results of the most promiscuous epitopes that show the best binding affinity

Epitope	Binding MHC molecule	Binding Energy (* ΔG kcal/mol)
AVHEALAPI	HLA A*02:06	-15.8010
KYFKRMAAM	HLA A*02:06	-20.5935
QTSNGGAAY	HLA A*02:06	-30.5467
RMAAMNQWL	HLA A*02:06	-20.6392
YFKEHGEPL	HLA A*02:06	-16.7505
IRGSIAAAH	HLA-DRB1*01:01	-20.6557
LFSSHMLDL	HLA-DRB1*01:01	-25.5732
VVAALEAAR	HLA-DRB1*01:01	-19.8404

*Global energy, it is the energy required to estimate the strength of association between the epitope within the active cleft of MHC molecule; more negative value indicates favored affinity and stable binding of the complex.

Figure 10. Illustrate the 2D interaction of the best docking poses of AVHEALAPI in the binding sites of HLA A*02:06.

Figure 11. Illustrate the 3D interaction of the best docking poses of KYFKRMAAM in the binding sites of HLA A*02:06.

Figure 12. Illustrate the 2D interaction of the best docking poses of QTSNGGAAY in the binding sites of HLA A*02:06.

Figure 13. Illustrate the 2D interaction of the best docking poses of RMAAMNQWL in the binding sites of HLA A*02:06.

Figure 14. Illustrate the 3D interaction of the best docking poses of YFKEHGEPL in the binding sites of HLA A*02:06.

Figure 15. Illustrate the 3D interaction of the best docking poses of IRGSIAAAH in the binding sites of HLA-DRB1*01:01.

Figure 16. Illustrate the 3D interaction of the best docking poses of LFSSHMLDL the binding sites of HLA-DRB1*01:01.

Figure 17. Illustrate the 2D interaction of the best docking poses of VVAALEAAR in the binding sites of HLA-DRB1*01:01.

4. Discussion:

In the present study, we predicted the most conserved and immunogenic B- and T-cell epitopes from Fba1 protein of *C. Glabrata* by using immunoinformatics approach in order to develop an effective epitope-based vaccine against this fungal pathogen which has emerged in recent years as serious health problem especially among immunosuppressed and hospitalized patient [79]. In a previous study conducted by *de Klerk et al*[80], showed that the Fba1 protein has the ability to provoke an immune responses in human against *M. mycetomatis*[80]. Also, several of recent publications have used the Fba1 protein as a strong antigenic target for predicting B- and T- cells epitopes in order to design promising vaccines against fungal and bacterial pathogens by using in silico tools such as *M. mycetomatis*, *P. Aeruginosa*, *L. Monocytogenes*, and *S. Mansoni* [81-84]. Hence there are more studies to explore Fructose bisphosphate aldolase protein immunogenic role and the possibility to find common conserved epitopes for different organisms.

The principle of using a cocktail of B- and T-cell epitopes in the epitope-based vaccine to trigger humoral as well as cellular mediated immune responses is much promising to

clear infection than humoral or cellular immunity alone, and it was applied before to enhance protection against different kind of infectious diseases ^[85, 86]. In this study, the analysis of Fba1 protein revealed 11 effective epitopes for B cell (AYFKEH, VDKESELYTK and HVDKESELYTK) and T cell (AVHEALAPI, KYFKRMAAM, QTSNGGAAY, RMAAMNQWL, YFKEHGEPL, IRGSIAAAH, LFSSHMLDL and VVAALEAAR). However, the molecular docking, which evaluates the binding affinity to MHCs molecules ^[51, 52], showed that the peptides QTSNGGAAY and LFSSHMLDL are the best candidates for designing an effective epitope-based vaccine against *C. galabrata*.

After retrieving the various sequences of *C. glabrata* Fructose bisphosphate aldolase protein, the protein reference sequence was submitted to Bepipred linear epitope prediction test, Emini surface accessibility test and Kolaskar and Tongaonkar antigenicity test in the IEDB, to determine the affinity of B cell epitopes and their position regarding the surface and their immunogenicity. Three peptides have passed (AYFKEH, VDKESELYTK and HVDKESELYTK) in all the prediction tests (Table I, II) (Figure 2-4). However, the MHC I binding prediction tool using Artificial neural network (ANN) ^[61] with half-maximal inhibitory concentration (IC₅₀) ≤ 500 revealed 114 conserved peptides interacting with various MHC I alleles. Three peptides were noticed to have the highest affinity in corresponding to their interaction with MHC I alleles. The peptide YIRSIAPAY from 93 to 101 had the affinity with 8 alleles to interact with (HLA-A*26:01,HLA-A*29:02,HLA-B*15:01,HLA-A*30:02,HLA-B*15:02,HLA-B*35:01,HLA-C*14:02,HLA-C*12:03), followed in order by KYFKRMAAM from 160 to 168 which interacts with 7 alleles (HLA-A*24:02,HLA-A*31:01,HLA-A*30:01,HLA-B*14:02,HLA-C*07:02,HLA-C*14:02,HLA-C*12:03) and QTSNGGAAY from 61 to 69 interacts with 7 alleles (HLA-A*01:01,HLA-A*26:01,HLA-A*30:02,HLA-A*29:02,HLA-B*15:02,HLA-B*15:01,HLA-B*35:01) (Table IV). While MHC II binding prediction tool using (NN-align) ^[67] with half-maximal inhibitory concentration (IC₅₀) ≤ 100 revealed 102 conserved peptides that interact with various MHC II alleles. Two peptides (LFSSHMLDL and YIRSIAPAY) were noted to have the highest affinity in corresponding to their interaction with MHC II alleles, both had the affinity to interact with 9 MHC II alleles (Table V).

Moreover, the predicted epitopes which have the high affinity to interact with MHC I, MHC II and combined MHC I with MHC II international alleles were analyzed by population coverage resource in the IEDB ^[68]. The population coverage of the five most promising epitopes (AVHEALAPI, KYFKRMAAM, QTSNGGAAY, RMAAMNQWL and YFKEHGEPL) for MHC I alleles was 92.54%, while for the three epitopes (IRGSIAAAH, LFSSHMLDL and VVAALEAAR) that showed high affinity to MHCII alleles was 99.58% throughout the world according to IEDB database (Figures 6-7). It should be noted that the population coverage of the five most promising epitopes that exhibited binding affinity to both MHC I and MHC II alleles (IAPAYGIPV, AAFGNVHGV, VVAALEAAR, YIRSTIAPAY and YQAGMVVLS) was 98.50% globally (Figure 8). However, the molecular docking revealed the epitopes QTSNGGAAY and LFSSHMLDL have high binding energy to MHC molecules HLA A*02:06 and HLA-DRB1*01:01, respectively which indicate favored affinity and stability in the epitope-molecule complex (Table VII) (Figure 10-17). This study was limited by being strictly computational; and more in vitro and in vivo studies to prove the effectiveness of the proposed peptides are highly recommended.

In conclusion

The epitope-based vaccines predicted by using immunoinformatics tools have remarkable advantages over the conventional vaccines that they are more specific, less time consuming, safe, less allergic and more antigenic. Further in vivo and in vitro experiments are needed to prove the effectiveness of the best candidate's epitopes (QTSNGGAAY and LFSSHMLDL). To the best of our knowledge, this is the first study that has predicted B- and T- cells epitopes from Fba1 protein by using in silico tools in order to design an effective epitope-based vaccine against *C. galabrata*.

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Conflict of interest:

The authors declare that there are no conflicts of interest.

Supplementary material

All data are included in this published article.

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