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In-silico Prediction of T and B cell Epitopes in the Evolutionary Conserved Pathway of Glycolysis for Human Pathogens: *Coccidiodes immitis, Histoplasma capsulatum and Pneumocystis carnii*

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Abstract

Fungal diseases are amongst the emerging diseases to which humans are most susceptible pertaining to the present day life style. New drugs are being made but at a slow pace, not matching the resistance developing capacity of the fungal pathogens. Therefore, it is important to choose new drug targets peculiar to fungi but absent in humans.A common practice till date has been to use the virulence proteins in order to devise medicines against micro-organisms. However, we have used *in-silico* techniques to analyze enzymes involved in the evolutionary conserved pathway of glycolysis which is the most primitive pathway for ATP production in aerobic as well as anaerobic organisms. Therefore, on successfully targeting these enzymes microorganism can be killed. We have chosen 3 fungal pathogens viz.*Coccidiodes immitis, Histoplasma capsulatum and Pneumocystis carnii* that cause serious diseases in human beings and their effect on the morbidity and mortality of humans has been substantial. MEME-Motif discovery tool was used to devise a single motif sequence per glycolytic enzyme from the three fungi. Further, online servers such as ABCpred, Bcepred, BepiPred and Tools from Immunomedicine group have been used in order to predict the linear epitopes from the motif sequences of the different glycolytic enzymes. Analysis of these epitopes was performed through PepCalc and NetSurfP in terms of parameters such as hydrophilicity, coil forming residues and exposed residues. Analysis of ten proteins of glycolysis from each fungus reveals the regions that can elicit an immunological response. This study most importantly projects aldolase to be an enzyme of importance which in future can be used as a potential vaccine target for these three fungi.

Keywords: *In-silico*, Epitope, Motif, Glycolysis, Fungi.

Introduction

In the recent past, there has been observed a massive increase in the human pathogenic diseases. The reason for the same can be attributed to the changing lifestyle as well as the modern day technologies (1). In-spite of the tremendous advancement of the medical facilities, these diseases are likely to follow an exponential expansion in the near future, reason being the fast pace at which the disease causing microorganisms are gaining resistance to the currently available drugs (1).

Therefore, the need of the hour is to find out new drug targets against these pathogens and develop suitable therapeutics for the same. The situation is graver when it comes to anti-mycotic drugs. The number of efficient drugs against the pathogenic fungi is very limited and those that are available are not free from side effects (2). The reason for the same is that fungi are eukaryotic organisms and have a lot in common with the human body cells. Therefore, it is difficult to devise suitable targets that would only be present in fungi and not in humans. Most of the research in this area is focused on identifying the various virulence factors and then to target the same with a suitable

therapeutic agent (2). But, in the current study, the authors have tried using a completely different approach to counter three fungal pathogens (*Coccidiodes immitis*, *Histoplasma capsulatum and Pneumocystis carnii*)by using the enzymes present in the evolutionary conserved pathway of glycolysis to serve as suitable antifungal drug targets. The reason behind choosing these fungal pathogensis that in the recent past a mass increase has been observed in the morbidity and mortality amongst the humans and the animals due to them (3). It is thus important to understand the pathology of these fungal organisms before stepping towards the immunoinformatic part of the manuscript.

Coccidiodes immitis*: Coccidiodes immitis* is a fungal pathogen responsible for causing valley fever or coccidioidomycosis. It is an endemic resident to the soil of south western United States, some parts of Mexico and Central and South America. It has a high prevalence due to its mode of infection. Its spores are present in the air and inhalation causes them to enter the lungs where the primary infection takes place (4). The general symptoms of the disease make it very difficult to distinguish from the other pulmonary diseases. Symptoms include fever, sore throat, cough, rashes on upper body and legs, night sweats, joint pain, headache, fatigue, and pleuritic chest pain etc. Generally, the body is able to ward off the infection by itself but in some cases medication is needed. A 3-6 months of fluconazole or any other antifungal administration will be enough to treat the disease. A recent study portraits the fact that 3089 coccidioidomycosis associated deaths have occurred in United States from 1990 to 2008 which accounts for about 200 deaths per year. This emphasizes the need of a suitable medicine to treat the fungus (http://www.cdc.gov/fungal/diseases/coccidioidomycosis/statistics.html; http://emedicine.medscape.com/article/215978-overview).

Histoplasma capsulatum: *Histoplasma capsulatum* is the causal organism of histoplasmosis. Large amounts of bat and bird droppings in a particular region make the soil and the area endemic to *Histoplasma*. It has been found in parts of America, Africa, Australia and Asia. Histoplasmosis generally occurs due to inhalation of aerial spores. Fever, cough, body ache, head ache, chills etc are some of the common symptoms of histoplasmosis (5). Itraconazoleis a drug of important therapeutic value in case of this disease and the course of the treatment may last from 3 months to about a year depending upon the severity of the symptoms. A study performed on patients hospitalized due to histoplasmosis reports that a mortality rate of 5% and 8% is associated with children and adults respectively (6).

Pneumocystis carnii: *Pneumocystis carnii* is the causal organism of a serious illness called Pneumocystis pneumonia (PCP). PCP can cause severe illness in people having weakened immune system. People with HIV/AIDS are more prone to this disease. The symptoms of this disease include fever, rapid breathing, cough etc. (https://www.nlm.nih.gov/medlineplus/ency/article/000671.htm). The best therapeutic option for this disease is Trimethoprim Sulfamethoxazole (TMP-SMX) which should be taken for three weeks. *Pneumocystic* pneumonia can cause lung failure hence is a life threatening disease. Nevertheless, the use of corticosteroids in people suffering from AIDS has decreased the amount of deaths (7). Amongst immunocompromised patients the mortality rates range from 5-40% in those who receive treatment whereas approaches 100% without treatment (http://www.cdc.gov/fungal/diseases/pneumocystis-pneumonia/statistics.html).

All of the above described fungi utilize glycolysis as the source for energy, precursor molecules and reducing power (in the form of NADPH). Glycolysis in general is a process in which glucose is converted into pyruvic acid by some enzyme catalyzed reactions. The utility of this pathway can be proved by the fact that it is ubiquitous in nature. Although this pathway has been deciphered decades before but very little is known about its regulation and control. This is the backbone of respiration and is of utmost

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importance for survival of both prokaryotes and eukaryotes.

There are slight variations amongst animals and fungi with respect to the enzymes that are utilized in glycolysis. For instance, two classes of aldolases have been found in nature. Class I aldolase is primarily present in plants and animals whereas class II aldolase is present in bacteria and fungi. Both aldolases utilize different reaction mechanisms, aldolase I requires Schiffs base as a reaction intermediate for the cleavage of fructose 1,6 bisphosphate whereas aldolase II makes use of Zn^{2+} in its active site (https://www.ebi.ac.uk/interpro/potm/2004_2/Page2.htm) for the same. Therefore, the authors have chosen this pathway for the identification of epitopes which can be targeted in order to devise suitable medicine against *C. immitis, H. capsulatum* and *P.carnii.*

Materials and Methods

Retrieval of the sequences of the respective genes from NCBI: The sequences of all the genes of the enzymes of glycolysis (Hexokinase, Phosphoglucoseisomerase, Phosphofructokinase, Fructose-bisphosphate-aldolase, Triosephosphate isomerase, Glyceraldehyde 3-phosphate dehydrogenase, Phosphoglycerate kinase, Phosphoglyceratemutase, Enolase and Pyruvate kinase) from the three organisms viz*Coccidiodes immitis, Histoplasma capsulatum and Pneumocystis carnii* have been retrieved from NCBI.

Discovery of the motif through MEME-Motif discovery tool: The sequences obtained from NCBI were subjected to the MEME- Motif discovery tool (http://meme-suite.org/). A single conserved sequence for all the three organisms for each gene was found out. The same process was repeated for all the enzymes of glycolysis.

***Mapping linear IgE binding epitopes*:** ABCpred (8), Bcepred (9), BepiPred (10) and Tools from Immunomedicine (11) group web servers were employed to predict linear B-cell epitopes. The web servers utilize the sequence of the protein as input and provide the possible epitopes as output based on the concepts of artificial neural networks, support vector machine, hydrophilicity scale with a hidden markov model, amino acid propensity scales of hydrophilicity, surface probability, antigenic index and flexibility (12).

***Property predicting softwares for the identified epitopes*:** Identified epitopes were analyzed with the help of 2 property predicting softwares viz. PepCalc (http://pepcalc.com/) and NetSurfP (http://www.cbs.dtu.dk/services/NetSurfP/). PepCalc analysed the epitopic sequences on the basis of parameters such as hydrophilic residues and polar residues whereas NetSurfP analyzed the epitope on the basis of the number of coil forming and the exposed residues.

Results

Retrieved sequences (Table-1) of different genes of glycolysis from *C.immitis, H.capsulatum and P. carnii* were subjected to MEME-Motif discovery tool and the obtained motif sequences of various glycolytic genes (Table 2) were used for epitope prediction.The individual results obtained from ABCpred, Bcepred, BepiPred and Tools from Immunomedicine group server have been provided in table 3, 4,5 and 6respectively. A single common epitope with the highest immunogenicity was chosen with the help of the mentioned webservers (Table-7).

Further, the identified epitopes were analyzed and the results of the same have been depicted in fig.1, which showed that the epitopes are high in the hydrophilic residues, coils and also

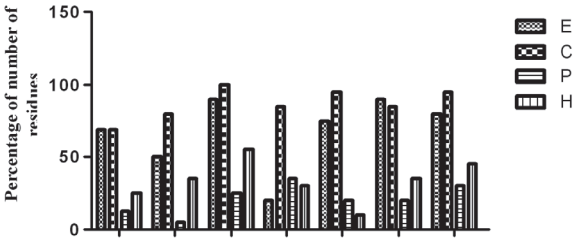

Fig. 1. A graph depicting the percentage of number of residues forming exposed (E), coiled(C), polar (P) and hydrophilic(H) regions in different glycolytic proteins. A:Hexokinase, B:Phosphofructokinase, C:Aldolase, D:Triosephosphateisomerase, E:Phosphoglycerate kinase, F: Enolase,G:Pyruvate kinase.

Table 1. Glycolytic genes and their respective IDs.

Glycolytic genes	*C. immitis*	*H.capsulatum*	*P. carnii*	*Homo sapiens*
Hexokinase	EAS35711.2	EDN07130.1	XP_007874379.1	AAC00173.1
Phosphoglucoisomerase	EAS29410.2	EDN04536.1	XP_007875677.1	P06744.4
Phosphofructokinase	EAS27971.2	EGC45399.1	XP_007872216.1	AAA60068.1
Fructose bisphosphatealdolase	3PM6	EGC40786.1	XP_007875432.1	P05062.2
Triose phosphate isomerase	P0CL22.1	EGC44423.1	XP_007874541.1	CAA49379.1
Glyceraldehyde-3-phosphate dehydrogenase	Q1DTF9.1	AAG33369.1	XP_007873850.1	CAA25833.1
Phosphpoglycerate kinase	EJB11848.1	EDN06855.1	XP_007874060.1	AAA60079.1
Phosphoglyceratemutase	EAS32175.2	EGC49880.1	XP_007875232.1	AAA60073.1
Enolase	EAS28926.2	EGC42819.1	XP_007874896.1	AAB59554.1
Pyruvate kinase	EAS29628.2	EGC42746.1	XP_007874297.1	AAA60104.1

Table 2. Motif sequences of the glycolytic genes obtained through the MEME-Motif discovery tool.

Name of the gene	Motif sequences
Hexokinase	INDTTGTLIASAYTDPEMRIGCIFGTGVNAAYMENAGSIPKIAHYN LPPDMPIAINCEY GAFDNERVVL
Phosphoglucoseisomerase	EVTKFGIDKRNMFGFESWVGGRYSVWSAIGLSVALYIGFDNFH QFLAGAHAMDKHFRETP LEQNIPVLGG
Phosphofructokinase	HRICEAVDEVFDTAASHQRGFVIEVMGRHCGWLALMSAISTGA DWLFIPE MPPRDGWEDD
Aldolase	SNIELTKRYLQRIAPMKQWLEMEIGITGGEEDGINNEEVSNNKL YTRPEDVF DVYSALSAISPYFSIAAAFGNVH
Triosephosphate isomerase	EHLRDDICVSAQNVYNSPPGPYTGEITVEQLKDAKILWTIV GHSERRIYFNESNQFIALK TKRALENGMS
Glyceraldehyde 3-phosphate dehydrogenase	ETYKSDIKVLSNASCTTNCLAPLAKVVNDNFGLVEGLMTTVHSY TATQKTVDGPSSKDWR GGRAAAQNII
Phosphoglycerate kinase	RKGLTALGDIYINDAFGTAHRAHSSMVGVNLPQRAAGFLVKK ELEYFAKALENPTRPFLA ILGGSKVSDK
Phosphoglyceratemutase	Common motif not found between the chosen sequences
Enolase	QACKLAQENGWGVMVSHRSGETEDTFIADLVVGLCTGQIKTGA PCRSERLAKYNQLMRIE EELGDEARFA
Pyruvate kinase	DEILDQADGVMVARGDLGIEIPAPKVFIAQKMMIAKCNIKGK PVICATQMLESMTYNPRP TRAEVSDVAN

Table 3. Epitopes for each glycolytic gene as predicted by ABCpred webserver along with their respective scores.

Glycolytic genes	Identified epitopes	Score
Hexokinase	DMPIAINCEYGAFDNE	0.95
	IGCIFGTGVNAAYMEN	0.91
	IASAYTDPEMRIGCIF	0.88
	AHYNLPPDMPIAINCE	0.84
	YMENAGSIPKIAHYNL	0.71
	TGVNAAYMENAGSIPK	0.69
Phosphoglucoseisomerase	KFGIDKRNMFGFESWV	0.83
	GFESWVGGRYSVWSAI	0.80
	YSVWSAIGLSVALYIG	0.70
	KHFRETPLEQNIPVLG	0.69
	GLSVALYIGFDNFHQF	0.69
	GAHAMDKHFRETPLEQ	0.64
	YIGFDNFHQFLAGAHA	0.56
Phosphofructokinase	AISTGADWLFIPEMPP	0.84
	HQRGFVIEVMGRHCGW	0.79
	WLFIPEMPPRDGWEDD	0.77
	GRHCGWLALMSAISTG	0.73
	RICEAVDEVFDTAASH	0.60
Aldolase	DEVFDTAASHQRGFVI	0.58
	MEIGITGGEEDGINNE	0.93
	EDGINNEEVSNNKLYT	0.82
	NNKLYTRPEDVFDVYS	0.80
	TKRYLQRIAPMKQWLE	0.64
	YSALSAISPYFSIAAA	0.64
	RIAPMKQWLEMEIGIT	0.51
Triosephosphate isomerase	LWTIVGHSERRIYFNE	0.95
	TGEITVEQLKDAKILW	0.88
	DICVSAQNVYNSPPGP	0.83
	ERRIYFNESNQFIALK	0.74
Glyceraldehyde 3-phosphate dehydrogenase	QKTVDGPSSKDWRGGR	0.90
	PSSKDWRGGRAAAQNI	0.88
	TYKSDIKVLSNASCTT	0.85
	LVEGLMTTVHSYTATQ	0.83
	ASCTTNCLAPLAKVVN	0.75
	CLAPLAKVVNDNFGLV	0.58
Phosphoglycerate kinase	GFLVKKELEYFAKALE	0.82
	FGTAHRAHSSMVGVNL	0.82
	PTRPFLAILGGSKVSD	0.71
	ELEYFAKALENPTRPF	0.64
	VGVNLPQRAAGFLVKK	0.56

Enolase	HRSGETEDTFIADLVV	0.92
	TGQIKTGAPCRSERLA	0.83
	ENGWGVMVSHRSGETE	0.76
	GAPCRSERLAKYNQLM	0.57
	ERLAKYNQLMRIEEEL	0.54
	DTFIADLVVGLCTGQI	0.53
	QLMRIEEELGDEARFA	0.52
Pyruvate kinase	GIEIPAPKVFIAQKMM	0.92
	EILDQADGVMVARGDL	0.82
	GVMVARGDLGIEIPAP	0.80
	PKVFIAQKMMIAKCNI	0.78
	ESMTYNPRPTRAEVSD	0.77
	MMIAKCNIKGKPVICA	0.72
	GKPVICATQMLESMTY	0.66

Table 4. Epitopes for each glycolytic gene as predicted by Bcepred webserver along with their respective scores.

Glycolytic genes	Identified epitopes	Score
Hexokinase	LINDTTGTLIASAYTDPEMR	0.805
Phosphoglucoseisomerase	None	
Phosphofructokinase	TGADWLFIPEMPPRDGWEDD	0.874
Aldolase	EIGITGGEEDGINNEEVSNN	0.999
Triosephosphateisomerase	VSAQNVYNSPPGPYTGEITV	0.998
Glyceraldehyde 3-phosphate dehydrogenase	None	-
Phosphoglycerate kinase	AHRAHSSMVGVNLPQRAAGF	0.789
Enolase	GLCTGQIKTGAPCRSERLAK	0.931
Pyruvate kinase	LESMTYNPRPTRAEVSDVAN	0.937

form the exposed regions. The maximum percentage of the residues forming the exposed regions i.e. 90% was in Aldolase and Enolase followed by Pyruvate Kinase (80%), Phosphoglycerate kinase (75%), Hexokinase (68.75%), Phosphofuctokinase (50%) and Triosephosphate isomerase (20%). Similarly, the highest percentage of the coil forming residues were also observed (100%) in Aldolase followed by Pyruvate kinase, Phosphoglycerate kinase, Enolase, Triosephosphate isomerase, Phosphofructokinase and Hexokinase with 95%, 95%, 85%, 85%, 80% and 68.75% respectively. Likewise, the maximum percentage of the residues forming the hydrophilic regions i.e. 55% was also in Aldolase followed by Pyruvate kinase (45%), Enolase (35%), Phsphofructokinase (35%), Triosephosphateisomerase (30%), Hexokinase (25%) and Phosphoglycerate kinase (10%).

Discussion

Three primary pathogenic fungi viz. Coccidiodes immitis, Histoplasma capsulatum and Pneumocystis carnii are responsible for increasing morbidity and mortality in the humans and animals. In the past some amount of work has been done in the field of epitope prediction and targeting with respect to non-glycolytic genes in the above

Table 5. Epitopes for each glycolytic gene as predicted by BepiPred webserver along with the score of each residue comprising the epitope.

Glycolytic genes	Identified epitopes along with the scores of each residue
Hexokinase	Y(0.802)T(0.966)D(1.040)P(0.618)E(0.529)M(0.352) M(0.467)E(0.530)N(0.478)A(0.557)G(0.755)S(0.759)I(0.575)P(0.385) N(0.596)L(0.751)P(0.879)P(0.689)D(0.854)M(0.383)P(0.514)
Phosphoglucoseisomerase	F(0.355)R(0.360)E(0.438)T(0.472)P(0.723)L(0.839)E(0.800)Q(0.472)
Phosphofructokinase	E(0.363)M(1.075)P(1.534)P(1.678)R(1.953)D(1.957)G(2.050) W(2.094) E (2.125)D(1.965)D(1.703)D(0.523)T(0.385)A(0.455)A (0.594)S(0.870) H(0.394)
Aldolase	G(0.534)I(0.826)T(1.041)G(1.148)G(1.533)E(1.328)E(1.698) D(1.681) G(1.692)I(1.626)N(1.258)N(1.106)E(0.994)E(1.005)V(1.271)S (1.220)N(1.186)N(1.091)
Triosephosphate isomerase	N(0.491)V(1.009)Y(1.350)N(1.760)S(1.913)P(1.924)P(2.284)G (2.458)P(2.443)Y(1.924)T(1.692)G(1.175)E(0.835)I(0.609)
Glyceraldehyde 3-phosphate dehydrogenase	Y(0.460)T(0.556)A(0.776)T(0.745)Q(1.006)K(1.308)T(1.422)V (1.690)D(1.842) G(1.847)P(1.965)S(1.690)S(1.889)K(1.706)D (1.626)W(1.552)R (1.312)G(1.048)G (0.936) R(0.814)A(1.030)A(0.614)
Phosphoglycerate kinase	G(0.516)T(0.390)A(0.294)H(0.032)R(0.547)L(0.481)E(0.954)N (1.011)P(0.781)T(0.529)R(0.539) K(0.801)V(1.172)S(1.224)D (1.197)K(1.088)
Enolase	I(0.663)K(0.793)T(0.824)G(0.916)A(0.846)P(1.098)C(1.028) R(0.674)R(0.6 30)S(1.056)G(1.495)E(1.533)T(1.280)E(0.948)D (0.669)T(0.556)E(0.630)L(0.721)G(1.018)D(0.611)E(0.456)
Pyruvate kinase	T(0.526)Y(1.023)N(1.205)P(1.363)R(1.482)P(1.562)T(1.511) R(1.408)A (1.367)E(1.0 18)V(0.812)S(0.722)D(0.562)V(0.633)A(0.514)N(0.691)

mentioned fungi. For instance, Antigen 2 (Ag2) has been identified as a major component of the mycelium and spherule based cell wall that can elicit T-cell responses in case of *Coccidioides* immune mice (13). Herr et al. (2007) (14) reported the presence of 6 epitopes (1P6:TRLTDF KCHCSKPELPGQIT; 1P7:HCSKPELPG QITPC VEEACP; 1P12: PIDIPPVDTTAAPE-PSETAE; 1P13: TTAAPEPSETAEPTAEPTEE; 1P15: PTEE PT AEPTAEPTAEPTHE and1P16: PTAEPTA EPTHEPTEEPTAV) in the Antigen 2 (Ag2)/Pra and the presence of 3 epitopes in (2P6: EKLTDF KCHCAKPELPGKIT; 2P13: DTRTPTQP PSTSPSAPQPTA; 2P14: PSTSPSAPQPTACI-PKRRRA) in PrP2 antigen. Similarly, Hurtgen et al (2012) (15) reported the presence of 2 antigens (P1: MRNSILLAATVLLGCTSAKVHL and P2: HVRALGQKYFGSLPSSQQQTV) in Pep1 antigen. In another study,6 antigenic epitopes of PRA viz. ARISVSNIV, GRPTASTPA, ALNLFVEAL, LVAAGLASA, FSHALJALV, AEPTHEPTE were identified so that the future therapeutics could be

Table 6. Epitopes for each glycolytic gene as predicted by Tools from Immunomedicine group webserver along with theaverage antigenic propensity score for each protein.

Hexokinase (Average antigenic propensity for this protein is 1.0173)	GTLIASA MRIGCIFG SIPKIAHYNL IAINCEY
Phosphoglucoseisomerase (Average antigenic propensity for this protein is 1.0226)	VGGRYSVWSAIGLSVALYIG NFHQFLAG
Phosphofructokinase (Average antigenic propensity for this protein is 1.0229)	CEAVDEVFDTAASHQRGFVIEVMG HCGWLALMS
Aldolase(Average antigenic propensity for this protein is 1.0075)	LTKRYLQR DVFDVYSALSAISPYFSIAAA
Triosephosphate isomerase(Average antigenic propensity for this protein is 1.0169)	RDDICVSAQNVYNSPPG KDAKILWTIVG QFIALKT
Glyceraldehyde 3-phosphate dehy drogenase (Average antigenic propensity for this protein is 1.0243)	SDIKVLSNA CTTNCLAPLAKVVNDNFGLVEGLMTTVHSY
Phosphoglyceratekinase(Average antigenic propensity for this protein is 1.0291)	HSSMVGVNLPQRAAGFLVKKELEYFAK TRPFLAILGG
Enolase(Average antigenic propensity for this protein is 1.0163)	WGVMVSH FIADLVVGLCTG KTGAPCRS
Pyruvate kinase(Average antigenic propensity for this protein is 1.0289)	DGVMVAR LGIEIPAPKVFI AKCNIKGKPVICAT

targeted against them (16). In case of *H. capsulatum*, epitopes have been reported on glycoproteins H,M and C antigens.They are common in major genera of dimorphic fungi and reported to be cross reactive galactomannan (17). A study made on the M antigen by using Jamenson-Wolf algorithm (Protean program, DNASTAR Inc, Madison, Wis., USA) reported that a large number of epitopes were found between amino acid 212 to 442 (18). Scheckelhoff and Deepe (2012) (19) observed the presence of an epitope (DGGQG) in the antigenic CDR3 fragment. Wang et al. (20) performed epitope mapping in *P. marneffei* and suggested that Mp1p, a cell wall mannoprotein can be used as a biomarker for its diagnosis and can also be targeted for therapeutic applications.

In the present study, the motif regions have been identified amongst all the three microorganisms for the proteins required in the glycolysis. And these motif sequences were further used for predicting the linear epitopes suggesting that aldolase amongst all the glycolytic enzymes can prove to be a fruitful

Table 7. Epitopes found from the motif sequences of the genes through ABCpred, Bcepred, BepiPred and Tools from Immunomedicine group.

Hexokinase	IASAYTDPEMRIGCIF
Phosphoglucoseisomerase	No epitope with suitable immunogenicity could be found
Phosphofructokinase	TGADWLFIPEMPPRDGWEDD
Aldolase	EIGITGGEEDGINNEEVSNN
Triosephosphate isomerase	VSAQNVYNSPPGPYTGEITV
Glyceraldehyde 3-phosphate dehydrogenase	No epitope with suitable immunogenicity could be found
Phosphoglycerate kinase	AHRAHSSMVGVNLPQRAAGF
Phosphoglyceratemutase	Common motif not found between the chosen sequences
Enolase	GLCTGQIKTGAPCRSERLAK
Pyruvate kinase	LESMTYNPRPTRAEVSDVAN

target for the future drugs against *C.immitis, H.capsulatum* and *P. carnii.* Mor et al. (21) suggested that aldolase can be used as a target antigen in case of Alzhiemer's disease.Similarly, in another study on cancer cells provides the insight that the aldolase is responsible for reduction in the cell proliferation by 90% utilizing a non-metabolic approach (22). Henceforth, aldolase is not only responsible for carrying out the glycolysis and production of ATP but it is also related to the cancer pathway in some way or the other. Furthermore, a study conducted by McCarthy et al. (23) on *Onchocerca volvulus* suggests thataldolase of this parasite can act as a vaccine candidate. Similarly, another study aiming to identify the target of the autoantibodies produced in patients suffering from hyperkinetic movement disorders (HMD) deciphered that glycolytic enzyme aldolase A was the target of those autoantibodies (24). Thus, from the above account it is clear that aldolase is not only useful in the glycolytic pathway but it has many roles in multiple areas of targeting.

Conclusion

A number of conclusions can be drawn from this study. First and the foremost is that glycolysis being the universal pathway can be targeted by the drugs for killing the micro-organisms. Secondly, a number of bioinformatics tools are available which can be used for determining the motif regions as well as the epitopic regions present in a sequence. Thirdly, the salient features of the epitopes are that they are rich in the residues which are hydrophilic in nature, are efficient in the formation of coils and also form the exposed regions. Fourthly, aldolase is most probable enzyme which can be used to determine epitopes and further usage as vaccine candidate against *Coccidiodes immitis, Histoplasma capsulatum* and *Pneumocystis carnii.*

Conflict of Interest

None

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References

1. Neelabh, Singh, K. and Rani, J. (2016). Sequential and Structural Aspects of Antifungal Peptides from Animals, Bacteria and Fungi Based on Bioinformatics Tools. Prob. Antimicrob. Prot, 8: 85-101.
2. Seneviratne, C. J. and Rosa, E. A. (2016). Editorial: Antifungal drug discovery: New theories and new therapies. Front. Microbiol. 7.
3. Baddley, J. W. (2011). Geographic Distribution of Endemic Fungal Infections among Older Persons, United States1-Volume 17, Number 9-Emerging Infectious Disease journal-CDC.
4. Stevens, D.A., Mandell, G.L., Douglas Jr, R.G. and Bennett, J.E. (1990). Coccidioidesimmitis. Principles and practice of infectious diseases(3rd edition), Churchill Livingstone Inc., New York, USA, pp.2008-2017.
5. Cano, M.V. and Hajjeh, R.A. (2001). The epidemiology of histoplasmosis: a review. In:Sem. Resp. Inf., 16:109-118.
6. Chu, J.H., Feudtner, C., Heydon, K., Walsh, T.J. and Zaoutis, T.E. (2006). Hospitalizations for endemic mycoses: a population-based national study. Clin. Infect. Dis., 42:822-825.
7. Thomas Jr, C.F. and Limper, A.H. (2004). Pneumocystis pneumonia. New Eng. J Med., 350: 2487-2498.
8. Saha, S. and Raghava, G.P.S. (2006). Prediction of continuous B cell epitopes in an antigen using recurrent neural network. Prot.: Str., Func.,Bioinfo., 65:40-48.
9. EL-Manzalawy, Y., Dobbs, D. and Honavar, V. (2008). Predicting linear B-cell epitopes using string kernels. J. Mol. Recog., 21:243-255.
10. Larsen, J.E.P., Lund, O. and Nielsen, M. (2006). Improved method for predicting linear B-cell epitopes. Immun. Res., 2: 2.
11. Kolaskar, A.S. and Tongaonkar, P.C. (1990). A semi empirical method for prediction of antigenic determinants on protein antigens. FEBS Lett., 276: 172-174.
12. Mishra, A., Jain, A. and Arora, N. (2016). Mapping B cell epitopes of major and minor peanut allergens and identifying residues contributing to IgE binding. J. Sci. Food Agr., 96: 539-547.
13. Zhu, Y., Tryon, V., Magee, D.M. and Cox, R.A. (1997). Identification of a *Coccidioides immitis* antigen 2 domain that expresses B-cell-reactive epitopes. Infect. Immun. 65:3376-3380.
14. Herr, R.A., Hung, C.Y. and Cole, G.T. (2007). Evaluation of two homologous proline-rich proteins of *Coccidioides posadasii* as candidate vaccines against coccidioidomycosis. Infect. Immun.75:5777–87.10.1128/IAI.00807-07.
15. Hurtgen, B.J., Hung, C.Y., Ostroff, G.R., Levitz, S.M. and Cole, G.T. (2012). Construction and evaluation of a novel recombinant T cell epitope-based vaccine against coccidioidomycosis. Infect. Immun. 80:3960–74.10.1128/IAI.00566-12.
16. Saxena, P. and Christi, J. (2014). Epitope Prediction and Structural Analysis of Pra Antigen of Coccidioides. Int. J. Pharm. Pharmaceut. Sci. 6:238-243.
17. Zancope-Oliveira, R.M., Bragg, S.L., Reiss, E. and Peralta, J.M. (1994). Immuno-chemical analysis of the H and M glyco-proteins from *Histoplasma capsulatum*. Clin Diag Lab Immunol, 1:563-568.
18. Guimarães, A.J., Hamilton, A.J., de M Guedes, H.L., Nosanchuk, J.D. and Zancopé-Oliveira, R.M. (2008). Biological Function and Molecular Mapping of M Antigen in Yeast Phase of *Histoplasma capsulatum*. PLoS ONE 3: e3449.
19. Scheckelhoff, M. and Deepe, G.S. (2002). The protective immune response to

heat shock protein 60 of *Histoplasma capsulatum* is mediated by a subset of V{beta}8.1/8.2+ T cells. J. Immunol. 169:5818–5826.

20. Wang, Y.F., Cai, J.P., Wang, Y.D., Dong, H., Hao, W., Jiang, L.X., Long, J., Chan, C., Woo, P.C., Lau, S.K. and Yuen, K.Y. (2011). Immunoassays Based on *Penicillium marneffei* Mp1p Derived from *Pichia pastoris* Expression System for Diagnosis of Penicilliosis. PLoS ONE 6(12): e28796.
21. Mor, F., Izak, M. and Cohen, I.R. (2005). Identification of aldolase as a target antigen in Alzheimer's disease. The J. Immunol., 175: 3439-3445.
22. Lew, C.R. and Tolan, D.R. (2012). Targeting of several glycolytic enzymes using RNA interference reveals aldolase affects cancer cell proliferation through a non-glycolytic mechanism. J. Biol. Chem., 287: 42554-42563.
23. McCarthy, J.S., Wieseman, M., Tropea, J., Kaslow, D., Abraham, D., Lustigman, S., Tuan, R., Guderian, R.H. and Nutman, T.B. (2002). Onchocerca volvulus glycolytic enzyme fructose-1, 6-bisphosphate aldolase as a target for a protective immune response in humans. Infect. Immun. 70: 851-858.
24. Privitera, D., Corti, V., Alessio, M., Volontè, A., Lampasona, V., Comi, G., Martino, G.,Franciotta D., Furlan, R., Fazio, R. (2013) Proteomic identification of aldolase A as an autoantibody target in patients with atypical movement disorders. Neurol. Sci., 34: 313-320.
25. Web references - http://meme-suite.org/, http://www.epitoolkit.de/, http://www.vls3d.com/links/bioinformatics/peptides-antibodies-epitopes, http://tools.immuneepitope.org/main/tcell/, http://osddlinux.osdd.net/vacci.php