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Computational Prediction of B-cell Epitopes of *Mycobacterium tuberculosis*-Implications in Vaccine Design

Mycobacterium tuberculosis'in B Hücresi Epitoplarının Hesaplamalı Tahmini-Aşı Tasarımındaki Etkileri

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Abstract

Introduction: Tuberculosis (TB) is a communicable disease caused by *Mycobacterium tuberculosis*. Bacillus Calmette-Guérin is the only vaccine available for TB. However, although the vaccine effectively protects children from TB, its efficacy in adults is still debatable. No effective vaccine is presently available to prevent TB. An effective vaccine should provoke humoral immunity to prevent the adhesion of *M. tuberculosis* to macrophages. In this context, B-cell epitopes may play an important role in vaccine development. Hence, this study aimed to identify B-cell epitopes using *in silico* tools.

Materials and Methods: In this study, B-cell epitopes were predicted using two tools (ABCPred and BCPREDS), which consists of three methods (artificial neural networks, BCPred, and AAP). Further, the epitopes predicted by the three prediction methods were analyzed for overlapping, and the ToxinPred, VaxiJen and AllerTop servers were used for analysis.

Results: A total of 2003 epitopes were predicted using all the prediction methods. Among these, 80 epitopes were predicted as overlapping epitopes, and 80, 57, and 29 epitopes were screened using the ToxinPred, VaxiJen, and AllerTop tools, respectively.

Conclusion: The epitopes predicted in the current study needs to be further validated using *in vitro* and *in vivo* analyses for B-cell response toward infection by *M. tuberculosis*.

Keywords: Epitopes, B-cell, *Mycobacterium tuberculosis*, vaccine

Öz

Giriş: Tüberküloz (TB), *Mycobacterium tuberculosis*'in neden olduğu bulaşıcı bir hastalıktır. Bacillus Calmette-Guérin, verem için kullanılabilen tek aşıdır. Bununla birlikte, aşı çocukları TB'den etkili bir şekilde koruduğu halde, yetişkinlerdeki etkinliği hala tartışmalıdır. Şu anda TB'yi önlemek için etkili bir aşı bulunmamaktadır. Etkili bir aşı, *M. tuberculosis*'in makrofajlara yapışmasını önlemek için humoral bağışıklığı tetiklemelidir. Bu bağlamda, B hücresi epitopları aşı geliştirmede önemli bir rol oynayabilir. Bu nedenle, bu çalışma *in silico* araçlar kullanılarak B hücresi epitoplarını tanımlamayı amaçlamıştır.

Gereç ve Yöntem: Bu çalışmada, B hücresi epitopları, üç yöntemden (yapay sinir ağları, BCPred ve AAP) oluşan iki araç (ABCPred ve BCPREDS) kullanılarak tahmin edildi. Ayrıca, üç tahmin yöntemiyle tahmin edilen epitoplar örtüşme açısından analiz edildi ve analiz için ToxinPred, VaxiJen ve AllerTop sunucuları kullanıldı.

Bulgular: Tüm tahmin yöntemleri kullanılarak toplam 2003 epitop tahmin edildi. Bunlar arasında 80 epitop, örtüşen epitoplar olarak tahmin edildi ve sırasıyla ToxinPred, VaxiJen ve AllerTop araçları kullanılarak 80, 57 ve 29 epitop tarandı.

Sonuç: Mevcut çalışmada tahmin edilen epitopların, *M. tuberculosis* enfeksiyonuna karşı B-hücresi tepkisine yönelik *in vitro* ve *in vivo* analizler kullanılarak doğrulanması gerekmektedir.

Anahtar Kelimeler: Epitoplar, B hücresi, *Mycobacterium tuberculosis*, aşı

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Introduction

Tuberculosis (TB) is a deadly infectious disease caused by *Mycobacterium tuberculosis*. It is an airborne pathogen that affects the lungs and causes pulmonary TB. One-third of the population worldwide is latently infected with *M. tuberculosis*[1,2]. It was estimated that 1.3 million deaths were recorded among human immunodeficiency virus (HIV)-negative people and 300,000 deaths in HIV-positive people. According to the World Health Organization, 10 million people were affected by TB in 2017 globally[3]. Bacillus Calmette-Guérin, a live attenuated strain of *Mycobacterium bovis*, is currently used as a vaccine in developing countries[4]. Although the vaccine confers protection against TB to children, its efficacy in adults is still controversial[5].

The activation of cell-mediated immunity (CMI) is considered essential for TB immunity, owing to the intracellular pathogenicity of *M. tuberculosis*[6]. Hence, most researchers have focused on the development of vaccines that induce CMI[7] since there is a limited role for humoral immunity against TB during active infection. In contrast, antibody-mediated immune responses against *M. tuberculosis* antigens have been studied for their role in the diagnosis of TB infections. Such studies rely on data supporting the correlation between pathogen load and serum antibody levels against *M. tuberculosis* antigens[8,9]. On the other hand, several experimental evidence has shown the modulation of the immune response to intracellular pathogens by humoral immunity[10-14]. Besides, humoral immunity-based vaccines with defensive efficacy were studied against several pathogens[15-19]. Hence, antibody-mediated immunity has been constantly emphasized as a significant element of defensive immune responses against *M. tuberculosis*[20]. The role of antibodies in host defense against *M. tuberculosis* has been reported in numerous studies[21-24]. Therefore, the administration of vaccines that stimulate *M. tuberculosis*-specific antibodies in the mucosa of the respiratory tract could be considered a successful approach. This may safeguard the host before *M. tuberculosis* enters the lungs and may prevent the primary infection. The neutralization of pathogens by antibodies was considered the most efficient antimicrobial vaccine in history[25].

Several restrictions are placed in conventional vaccines that are either prepared by attenuation or inactivation of the whole pathogen. One such restriction is the genetic variability of the pathogen worldwide, which diminishes vaccine efficacy in different parts of the world[26]. Computational algorithms are generally considered a backbone of immunoinformatic tools[27], and these tools make the method for screening potential epitopes faster and cost effective[28,29]. Our study aimed to identify novel B-cell candidates for an epitope-based TB vaccine using immunoinformatic tools.

Materials and Methods

Selection of Target Proteins

Based on the literature survey, 10 possible candidate vaccine proteins were retrieved from the National Center for Biotechnology Information database and used in this study. The list of proteins selected and their accession number is presented in Table 1[30].

B-cell Epitope Prediction

The B-cell epitope prediction was conducted using two different tools. The ABCPred tool (http://crdd.osdd.net/raghava/abcpred/) was used to predict B-cell epitopes based on artificial neural networks (ANN) algorithm[31]. On the other hand, the BCPREDS tool (http://ailab.ist.psu.edu/bcpred/) employs two methods (BCPred and AAP) to predict B-cell epitopes based on Support Vector Machine (SVM) algorithm[32,33]. The default prediction methods were chosen for all prediction methods.

Overlapping Epitope Prediction

The epitopes predicted by more than one tool are considered overlapping epitopes. Epitopes predicted by each tool, mentioned above, were manually compared with one another for the identification of overlapping epitopes.

Table 1. Targeted protein sequences selected for B-cell epitope prediction

Accession No	Gene	Protein
CCP46633.1	FbpA	Mycolyl transferase 85A (fibronectin-binding protein A) (antigen 85 complex A)
CCP44652.1	FbpB	Mycolyl transferase 85B (fibronectin-binding protein B) (antigen 85 complex B)
CCP46704.1	EsxA	6 kDa early secretory antigenic target EsxA (ESAT-6)
CCP43018.1	EsxH	Low molecular weight protein antigen 7 EsxH (10 kDa antigen) (CFP-7) (protein TB10.4)
CCP46442.1	EsxV	Putative ESAT-6 like protein EsxV (ESAT-6 like protein 1)
CCP46443.1	EsxW	Putative ESAT-6 like protein EsxW (ESAT-6 like protein 10)
CCP43952.1	PPE18	PPE family protein PPE18
CCP45405.1	PPE42	PPE family protein PPE42
CCP42850.1	PepA	Probable serine protease PepA (serine proteinase) (MTB32A)
CCP45458.1	Rv2660c	Hypothetical protein Rv2660c

Prediction of Nontoxic Epitopes

The predicted overlapping epitopes were further analyzed using ToxinPred v2.0 server (https://webs.iiitd.edu.in/raghava/toxinpred/index.html) for the prediction of toxic peptides[34]. Peptides predicted as toxic were excluded, and nontoxic peptides were used for further analysis.

Prediction of Antigenic Epitopes

The nontoxic peptides were further evaluated using VaxiJen v2.0 (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) server for the identification of antigenic peptides[35]. The default threshold value of 0.4 was selected for antigenicity prediction. The nonantigen peptides were excluded, and the peptides with above the threshold values were considered further as potential vaccine candidates.

Identification of Nonallergenic Peptides

Antigenic peptides predicted by VaxiJen v2.0 tool were further analyzed using AllerTop v.2 tool (https://www.ddg-pharmfac.net/AllerTOP/index.html) for the prediction of "probable allergen" or "probable nonallergen." The default threshold value is selected for this analysis[36].

As the work does not involve animals or human patients, ethical committee approval and patient consent form are not indicated.

No statistical analysis was conducted in this study, and hence, that is not provided.

Results

B-cell Epitope Prediction

A total of 2003 B-cell epitopes (12 mer to 20 mer) were predicted using the three methods with two different tools. ABCPred predicted the highest number of epitopes with 1323, followed by BCPred and AAP methods with 353 and 327 epitopes, respectively. PPE42 protein (439) exhibited the highest number of epitopes, followed by PepA (382), PPE (306), FbpA (300), FbpB (259), EsxA (70), EsxV (69), EsxH (64), and EsxW (63). The lowest number of epitopes was predicted in the hypothetical protein Rv2660c with 51 (Figure 1).

Overlapping Epitope Prediction

A total of 80 overlapping epitopes of varying lengths were identified. The highest number of epitopes was predicted in 12 mer and 16 mer (20 each), followed by 14 mer (16), 18 mer (14), and 20 mer (10; Figure 2).

Prediction of Nontoxic Epitopes

Predicted overlapping epitopes were further analyzed using ToxinPred tool for prediction of toxic peptides. A total of 80 epitopes were analyzed, and none of the epitopes were predicted as toxic.

Prediction of Antigenic Epitopes

Antigenicity characteristics of the predicted epitopes were analyzed using VaxiJen tool. Of the 80 predicted epitopes, 57 were identified as "probable antigen." Among these, 15 epitopes were of 16 mer in length, which is 26.32% of the total epitopes analyzed in this study. This was followed by 21.5% (12), 19.30% (11), 17.54% (10), and 15.79% (9), and epitopes were predicted in 12 mer, 14 mer, 18 mer, and 20 mer, respectively (Figure 3).

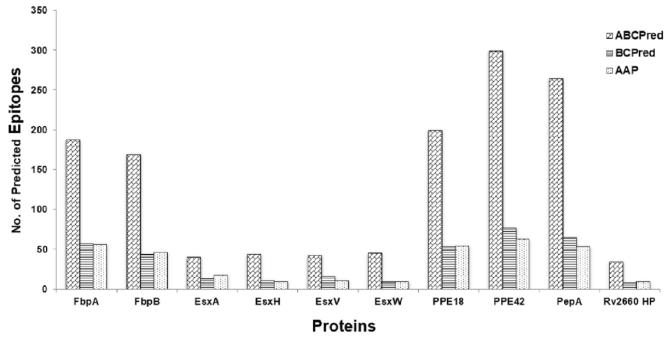

Figure 1. B-cell epitope prediction using the three different methods

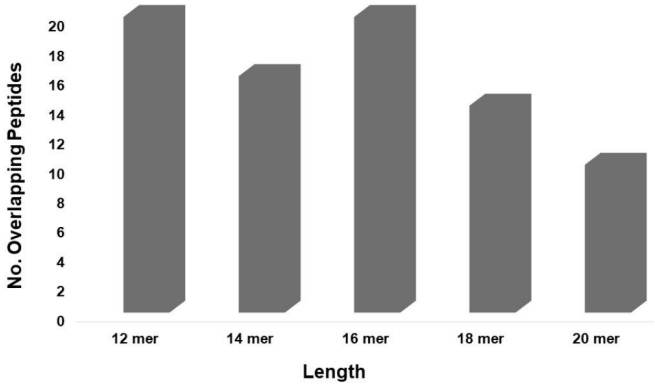

Figure 2. Predicting overlapping epitopes among different length peptides

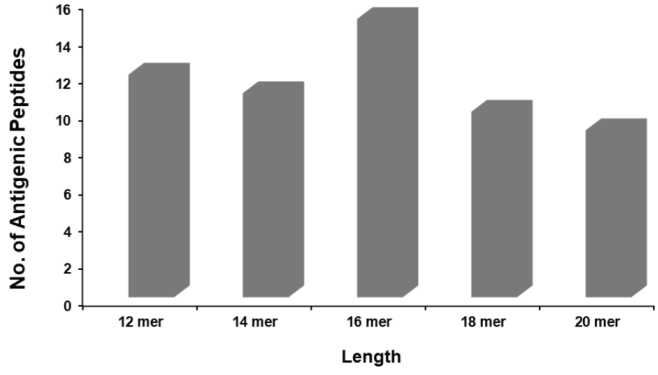

Figure 3. Antigenic epitopes identified among predicted overlapping epitopes

Identification of Nonallergenic Peptides

A total of 29 nonallergic epitopes were identified out of 57 antigenic peptides using AllerTop v.2 tool. Of epitopes, 24.14% (7) were identified as nonallergen with 20 mer in length, and 20.69% exhibited positive predictive value for 14 mer (6), 16 mer (6), and 18 mer (6). Only 4 epitopes were predicted as nonallergic peptide with 12 mer in length (Figure 4).

Discussion

Despite the years of research on the pathogenesis of TB, the development of a potential drug or vaccine that can eliminate the disease remains elusive. Various strategies are being developed toward a better TB vaccine that includes subunit, viral vector, inactivated whole-cell *Mycobacterium*, and DNA vaccines[30]. The current vaccine used for TB is not provoking a sufficient immune response in adults. Hence, there is a need for an effective vaccine that could prevent TB at various stages of infection[6,37]. Identifying B-cell epitopes that can induce a strong antibody response could contribute to the development of a novel vaccine candidate or could be added to the existing vaccine to boost the immune response. In this study, a reverse vaccinology approach was used to predict potent B-cell epitopes in major antigens of *M. tuberculosis*.

Recently, several computational algorithms based on SVM, ANN, and quantitative matrix (QM) have been developed and are widely used as epitope prediction tools[38]. These immunoinformatic approaches significantly reduce the time and effort taken to screen potential epitopes[28,29]. Studies have indicated that the antibodies produced against *M. tuberculosis* proteins could play a significant role in preventing the attachment of macrophages[39]. Therefore, identifying B-cell epitopes in *M. tuberculosis* could be a promising approach to initiate a protective immune response. Various immunoinformatic tools are available to predict potent B-cell epitopes. In this study, two B-cell epitope prediction tools, BCPREDS and ABCPred, were employed to analyze the immunogenic peptides that occur in

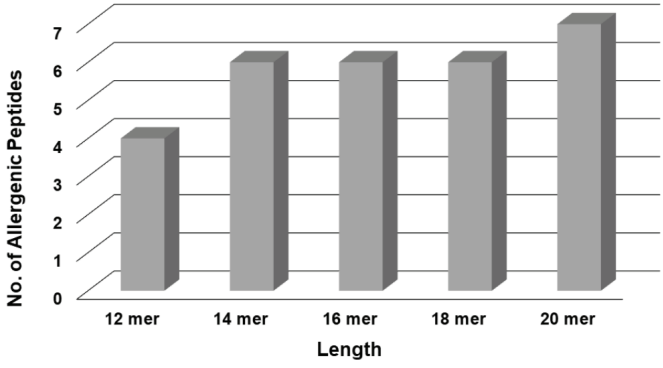

Figure 4. Nonallergic epitopes identified among antigenic epitopes

10 potent *M. tuberculosis* vaccine candidates. A total of 2003 peptides were identified using three B-cell epitope prediction methods.

Instead of using a single algorithm, a peptide predicted by more than one algorithm may increase prediction efficacy. Hence, peptides predicted by each algorithm were compared with one another to predict overlapping epitopes[40]. A total of 80 (3.99%) epitopes were identified as overlapping epitopes. Interestingly, the Rv2660c peptide GGVTVGVGVGTE$_{24-35}$ was predicted as a B-cell epitope by the three prediction methods, exhibiting a score of 1 in both BCPred and AAP methods.

The epitopes that are predicted should be nontoxic to the cells, and hence, they were screened for toxicity using ToxinPred server. Various properties of peptides are used to develop these methods based on the machine learning technique and QM[41]. Of the 80 overlapping epitopes analyzed, none of the epitopes were predicted as toxic.

An antigenic epitope would have an ability to bind with antibodies and cells of the immune system[42]. The antigenicity of the predicted epitopes was analyzed using VaxiJen v2.0 server. Of the 80 epitopes, 57 were predicted to be antigenic. The highest number of VaxiJen score was predicted for the FbpA peptide, SNIKFQDAYNAGGGHNGV$_{279-297}$ at 1.8452. A total of 15 epitopes were predicted by VaxiJen with a score of more than 1. Nonantigenic epitopes were eliminated from further analysis.

A good vaccine candidate should not cause an allergic reaction to the host system. Therefore, allergenicity prediction is a key step to prepare epitope-based vaccines. However, most of the vaccines cause allergic reactions with the help of immunoglobulin E and type 2 T-helper cells[43]. To overcome this problem, the allergic epitopes were identified through AllerTop v.2.0 server and excluded from further study. Of the 57 antigenic epitopes analyzed, 29 (50.88%) were predicted as nonallergens.

Finally, 29 immunodominant epitopes were identified from 7 of 10 *M. tuberculosis* protein candidates. All these epitopes have shown positive prediction scores and were found to be nontoxic, antigenic, and nonallergic. Incidentally, 3 of the 29 epitopes were already reported to be epitopes when analyzed *in vitro*. The EsxH peptide, YAGTLQSLGAEIAVEQAA$_{21-38}$, was reported as a T-cell epitope that binds to MHC class II alleles (HLA-DRB1)[44]. Meanwhile, the FbpA peptide PVGGQSSFYSDWYQPACGKA$_{114-133}$ was already identified as a T-cell epitope that can bind to HLA class II and involved in IFN-γ production[45].

The remaining 26 peptides are novel epitopes identified from the seven *M. tuberculosis* proteins. Among these, 8 epitopes (LTVPPPVIAENRAE$_{104-117}$, RVPPRPYVMPHSPA$_{376-389}$, YGLTVPPPVIAENRAE$_{102-117}$, RVPPRPYVMPHSPAAG$_{376-391}$, MVAAASPYVAWMSVTAGQ$_{66-83}$,

AAAMFGYAAATATATATL$_{149-166}$, GPGSASLVAAAQMWDSVASD$_{19-38}$, and YETAYGLTVPPPVIAENRAE$_{98-117}$) were present in the PPE18 protein. The PPE18 protein interacts with macrophages and activates IL-10, reducing host protective responses[46].

Ag85 is a complex protein that binds to fibronectin and elastin proteins. The main role of this protein is to prevent phagosome maturation in macrophages[47]. A total of 8 epitopes (5 from FbpA: FQSGGANSPALY$_{69-80}$, QDAYNAGGGHNGVFDF$_{284-299}$, AMGDAGGYKASDMWGPKEDP$_{210-229}$, PVGGQSSFYSDWYQPACGKA$_{114-133}$, and PDLQRALGATPNTGPAPQGA$_{319-338}$, and 3 from FbpB: VPSPSMGRDIKV$_{53-64}$, LDPSQGMGPSLI$_{193-204}$, and GDAGGYKAADMW$_{209-220}$) were identified in the Ag85 family of proteins. Another 3 epitopes were predicted in the EsxH protein (RAYHAMSSTHEANT$_{67-80}$, LVRAYHAMSSTHEANT$_{65-80}$, and YAGTLQSLGAEIAVEQAA$_{21-38}$) and 4 epitopes in the PPE42 (TTGLAGDAWHGPAS$_{49-62}$, GATPADAYPTVDYA$_{414-427}$, ASVTTGLAGDAWHGPASL$_{46-63}$, and YHSAASAVATQLAPIQEG$_{154-171}$). The EsxH protein interacts with the endosomal sorting complexes required for transport and thereby prevents phagosomal maturation and antigen presentation[48,49]. These novel peptides from various proteins of *M. tuberculosis* could be potential epitopes.

Although this study yielded novel putative epitopes, further *in vitro* and *in vivo* analyses need to be conducted before confirming the immunogenicity of the epitopes.

Conclusion

The reverse vaccinology approach used to predict antibody-eliciting B-cell epitopes has yielded 26 novel peptides that could be potent B-cell epitopes. These peptides could be further explored for their immunogenicity in animal models and could form a potential candidate to be included in vaccine preparation.

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Ethics

Ethics Committee Approval and Informed Consent: As the work does not involve animals or human patients, ethical committee approval and patient consent form are not indicated.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: E.T., M.M., K.S., Design: E.T., M.M., K.S., Data Collection or Processing: E.T., Analysis or Interpretation: E.T., K.S., Literature Search: E.T., Writing: E.T., K.S.

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