JKKI
SCIENTIA EST BASIC VITAE

JKKI: Jurnal Kedokteran dan Kesehatan Indonesia
Indonesian Journal of Medicine and Health
Journal homepage: https://journal.uii.ac.id/JKKI
P-ISSN 2085-4145 | E-ISSN 2527-2950

B-cell epitope prediction of MPB83 protein as a candidate for serodiagnostic antigen of bovine tuberculosis in human: In silico study

Shabrina Izzata Afiyati Ahmad[1], Ayu Munawaroh Aziz[2], Yunita Armiyanti[3], Diana Chusna Mufida*[4]
[1]Faculty of Medicine, University of Jember, Jember, Indonesia
[2]Department of Histology, Faculty of Medicine, University of Jember, Jember, Indonesia
[3]Department of Parasitology, Faculty of Medicine, University of Jember, Jember, Indonesia
[4]Department of Microbiology, Faculty of Medicine, University of Jember, Jember, Indonesia

Original Article

ARTICLE INFO

Keywords:
diagnostic tool,
serology,
MPB83 protein, B-cell epitope,
bioinformatics,
immune response

***Corresponding author:**
chusna.fk@unej.ac.id

DOI: 10.20885/JKKI.Vol15.Iss2.art4

History:
Received: November 13, 2023
Accepted: August 23, 2024
Online: August 27, 2024

ABSTRACT

Background: Bovine tuberculosis (bTB) can be transmitted to humans by inhalation or consumption of incomplete pasteurized milk and dairy products derived from infected cows. Most cases of *Mycobacterium tuberculosis* (*M. bovis*) infection are resistant to tuberculosis (TB) drugs. The risk of death during treatment for bTB has been reported to be 2.55 times higher than for TB. However, the quality of diagnostic methods for bTB remains relatively low.

Objective: We aim to evaluate the potential of the B-cell epitope of the MPB83 protein as a candidate bTB serodiagnostic antigen using an in silico approach.

Methods: This study was a computer-based descriptive study using secondary data from the National Center for Biotechnology Information (NCBI) protein database. The MPB83 protein sequence was obtained from *M. tuberculosis* variant bovis AF2122/97 from the United Kingdom. We described the characteristics of the linear epitope of the *M. bovis* B-cell protein MPB83 by measuring antigenicity, molecular weight, instability index, and Grand Average of Hydropathy (GRAVY) score. The tools used in this study were IBIVU PRALINE, VaxiJen v2.0, IEDB, ExPASy ProtParam, Cluspro, and the PyMOL application.

Results: We found an epitope that could be used for bTB serodiagnostic antigen with low conservation, the $_{106}$KLNPDVNLVDTLN$_{118}$ epitope. It has the molecular weight, instability index, and GRAVY score of 1638.76 Da, -28.44, and -0.300, respectively. Epitopes with the best criteria were simulated by docking to human major histocompatibility complex (MHC) class II. Docking results showed that the lowest binding energy was -644.8 kcal/mol. Further analysis using the PyMOL application obtained 14 hydrogen bonds with bond distances ranging from 1.7 Å to 2.2 Å, all of which showed strong hydrogen bonds.

Conclusion: The B-cell epitope of MPB83 protein sequence $_{106}$KLNPDVNLVDTLN$_{118}$ has a potential serodiagnostic antigen candidate for human bTB.

INTRODUCTION

Tuberculosis is a communicable disease caused by *M. tuberculosis*, which is still a world health problem with high mortality and morbidity rates.[1] Tuberculosis can be caused by other species from the Mycobacterium group, such as *M. bovis*, which causes bTB.[2,3] In 2016, cases of bTB on a global scale reached 147,000, with a death rate of 12,500.[4] The burden of bTB in developing countries in the human population is about 10%, with 2.1% pulmonary TB and 9.4% extrapulmonary TB cases.[5]

Bovine tuberculosis is a zoonotic disease with livestock as the primary host, especially dairy cows. *M. bovis* can infect humans directly through inhalation or non-intact skin. Milk and its processed products that are not entirely pasteurized can be a source of *M. bovis* excretion from infected cows.[6] Many cases of bTB were found to be resistant to several TB drugs, such as isoniazid, rifampicin, and pyrazinamide.[5] The resistance rate reaches 70% of all cases, and it is one of the reasons for the high rate of MDR-TB (Multidrug-resistant tuberculosis).[7,8] The risk of death for bTB during treatment was reported to be 2.55 times higher than TB.[3]

The quality of bTB diagnosis is still relatively low because it is carried out with the same clinical and microscopic examination as TB.[9] A specific diagnostic method needs to be developed to differentiate *M. bovis* infection from other species of the Mycobacterium group because its infection causes a higher risk of resistance, requiring further examination for the level of sensitivity of each drug. When several drugs are found to be resistant, the duration and type of drugs used will follow the management of drug-resistant TB.[7,8] Based on bTB studies conducted on livestock, *M. bovis* was reported to have an immunodominant antigen, the MPB83 protein. The MPB83 protein is a protein that is widely expressed by *M. bovis*.[10,11] Seroreactivity rates in response to IgG and IgM to MPB83 were reported to be 97.5% and to other M. bovis protein antigens only to be 85%.[10] MPB83 was used in recombinant form to investigate the effect of the protein on the immune response. They recruited the protein in vitro to the murine macrophage cell line RAW264.[7] The data showed that MPB83 is a glycosylated lipoprotein processed by type II signal peptidase II and located at the bacterial membrane, which confers more vital immunogenic properties when compared to MPB70, so it is important to note that MPB83 is an immunogenic antigen in *M. bovis* infection.[12]

Similar to the gold standard for diagnosing TB, bTB sputum culture takes a long time, is expensive, can only be performed by experts, and is not adequate for patients with extrapulmonary TB. Sensitive and specific PCR (Polymerase Chain Reaction) tests are impractical and expensive.[13] The serological test using protein antigen as a supporting examination is commonly used, namely tuberculosis immunochromatography (ICT), with a sensitivity that is still relatively low, at 35%.[14] This low sensitivity will increase the risk of false negatives, so developing diagnostic methods by combining several tests or increasing sensitivity is urgently needed.[14] A previous study attempted to increase serodiagnostic sensitivity by 5.1% to 5.8% by replacing the antigen protein with B-cell epitopes.[15] B-cell epitopes as amino acids can be accessed on the surface, recognized by secreted antibodies or directly on B-cell receptors, and elicit cellular or humoral immune responses.[16] Based on these studies, a serodiagnostic innovation by utilizing the MPB83 protein B-cell epitope that is more specific to *M. bovis* needs to be developed.

In silico characterization of one of the proteins from *M. bovis* species is the first step in designing a diagnostic method utilizing the MPB83 protein epitope. The results map the amino acid chain and its interactions, which can be used as a diagnostic tool.[15,17] These mapping results can reduce the failure rate in trial and error in subsequent validation studies by in vitro, in vivo, and clinical trials, as well as reduce the research costs effectively. The identification of the B-cell epitope of MPB83 protein using the in silico approach has just been performed. This study aims to assess the potential of the B-cell epitope of MPB83 protein as a candidate for bTB serodiagnostic antigen using an in silico approach.

METHODS

Study design

We performed computer-based descriptive research by using secondary data taken from the NCBI protein databank (https://www.ncbi.nlm.nih.gov/protein) to obtain MPB83 protein sequences from Mycobacterium tuberculosis variant bovis AF2122/97 originating from the United Kingdom. The accession number of this sequence in the NCBI Protein databank is P0CAX7. The AF2122/97 is a reference strain of the M. bovis species commonly used as a basis for research and the primary strain in vaccine development, diagnostics, and therapy development for bTB.[18,19] This study describes the characteristics of the B-cell epitopes of the MPB83 protein.

Data collection

The instruments used in the study were Asus® G5O4FLP laptop with Intel® Core™ i5-7200U CPU 2.50 GHz 16 GB RAM and Windows® 10 Pro 64-bit

operating system. The characterization was carried out using several online servers, such as IBIVU PRALINE (https://www.ibi.vu.nl/programs/pralinewww/), IEDB (http://tools.iedb.org/bcell/), VaxiJen v2.0 (https://www.ddgpharmfac.net/vaxijen), and ExPASy ProtParam (http://www.expasy.org/tools/protparam.html). The IBIVU PRALINE is an online server used to assess the conservation level of MPB83 protein. Epitope prediction was performed using the IEDB (Immune Epitope Database) with the Bepipred linear 2.0 method (threshold = 0.5), and the antigenicity of each epitope was tested with the VaxiJen v2.0 server (threshold = 0.4).[15,17] We analyzed the epitopes with antigenicity values that exceeded the threshold-based physicochemical properties using the ExPASy ProtParam server based on their physicochemical properties. Docking simulations were carried out to describe the binding of the best B-cell epitope MPB83 protein candidates to MHC class II using the ClusPro server (https://cluspro.bu.edu/login.php). The docking simulation was performed using blind docking, which is the docking of the ligand across the protein surface without understanding the target binding sites that might interact.[20] Furthermore, the docking results were visualized using the open-source PyMOL application (https://pymol.org/2/).

Ethics

Ethical clearance is not required for this research because this is a bioinformatics study, which does not require samples from living beings.

RESULTS

Protein sequence homology of MPB83

We aligned the MPB83 protein sequence of *M. bovis* strain AF2122/97 with the Ag85 protein sequence of the M. tuberculosis complex as a candidate antigen protein for TB biomarkers in M. tuberculosis infection. The *M. tuberculosis* complex Ag85 protein consists of Ag85A, Ag85B, and Ag85C proteins. Table 1 displays each sequence subjected to homology testing with the MPB83 sequence of M. bovis.

Antigenicity of MPB83 protein sequences

The results of the antigenicity test show that the MPB83 *M. bovis* protein is an antigenic protein because it has a score of 0.5742. The protein is considered antigenic because it has a score higher than the predetermined threshold value of 0.4 and is said to be non-antigenic if the score obtained is less than the predetermined threshold value.

Table 1. Alignment results of MPB83 protein of *Mycobacterium bovis* strain AF2122/97 with Ag85 protein sequence of Mycobacterium tuberculosis complex

Data	Spesies		
	Ag85A *M. tuberculosis* (strain H37Rv)	**Ag85B *M. tuberculosis* (strain H37Rv)**	**Ag85C *M. tuberculosis* (strain H37Rv)**
Percent sequence identity	0.32	0.27	0.28
Number of residues	558	545	560
Number of gaps	118	109	124

Prediction and antigenicity test of B-cell epitopes of *M. bovis* MPB83 protein

Figure 1A shows the results of predicting B-cell epitopes on the MPB83 protein sequence of *M. bovis*. Furthermore, the antigenicity test was carried out on the linear B-cell epitope sequences obtained using the VaxiJen v2.0 server and Kolaskar and Tongaonkar. These results are shown in Table 2 and Figure 1B.

Physicochemical test of B-cell epitopes of *M. bovis* MPB83 protein

The physicochemical test results showed that the $_{106}$KLNPDVNLVDTLN$_{118}$ epitope has the most potential to be used as a bTB serodiagnostic antigen protein. Epitope $_{106}$KLNPDVNLVDTLN$_{118}$ has a molecular weight of 1,454.64 Da, is stable, and hydrophilic, as shown in Table 3.

B-cell epitope binding analysis of MPB83 protein with MHC class II

We performed molecular docking using Cluspro to obtain results in binding energy and binding interaction model between epitope $_{106}$KLNPDVNLVDTLN$_{118}$ with MHC Class II (PDB ID: 1J8H). Interaction model number 3 of 14 existing

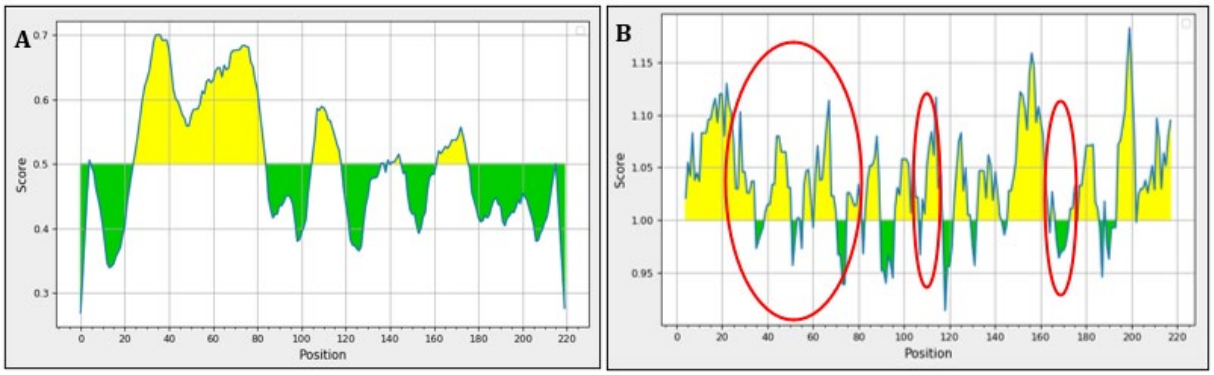

Figure 1. A. Linear epitope prediction results in MPB83 B-cells *M. bovis* strain AF2122/97. The yellow area results from epitope prediction, namely the area with a threshold exceeding the predetermined threshold of 0.5. B. Antigenicity test results of MPB83 B-cell linear epitopes of *M. bovis* strain AF2122/97 using Kolaskar & Tongaonkar Antigenicity.

Table 2. Prediction results and antigenicity test of linear epitopes of MPB83 B-cells of *M. bovis* strain AF2122/97 using VaxiJen v2.0

Name	Start	End	Peptide	Length	Antigenicity (T = 0,4)	
					Score	Interpretation
MBP83	5	5	Q	1	-	Non-Antigen
	25	84	CSSTKPVSQDTSPKPATSPAAPVTTAAMADP AADLIGRGCAQYAAQNPTGPGSVAGMAQD	60	0.4987	Antigen
	106	118	KLNPDVNLVDTLN	13	0.8693	Antigen
	137	138	AA	2	-	Non-Antigen
	140	140	I	1	-	Non-Antigen
	142	146	QLKTD	5	-	Non-Antigen
	162	176	QASPSRIDGTHQTLQ	15	1.0949	Antigen

Table 3. Results of physicochemical testing of linear epitopes of B-cell protein MPB83 of *M. bovis*

Name	No	Start	End	Molecular Weight (Da)	Instability Index		GRAVY score	
					Score	Interpretation	Score	Interpretation
MPB83	1	25	84	5,788.40	50.81	Unstable	-0.263	Hydrophilic
	2	106	118	1,454.64	-28.44	Stabilized	-0.300	Hydrophilic
	3	162	176	1,638.76	80.38	Unstable	-1.107	Hydrophilic

models showed the lowest binding energy value of -644.8 (kcal/mol) in the ClusPro score. Then, each hydrogen bond formed in model number 3 was analyzed for bond strength based on the bond distance in angular units (Å) shown in Table 4 and Figures 2A and 2B. Interactions formed at distances less than 3 Å indicate strong bonds.[21] The strong binding of epitope with MHC class II receptors indicates that the epitope can trigger an immune response like all *M. bovis* MPB83 protein antigens.

DISCUSSION

Our in silico characterization of the proteins from *Mycobacterium bovis* species, a crucial step in designing a diagnostic method utilizing the MPB83 protein epitope, has led to a promising discovery. The $_{106}$KLNPDVNLVDTLN$_{118}$ epitope, identified based on its characterization and molecular docking results, emerges as a potential candidate antigen for serodiagnostic for TB due to *M. bovis*. Notably, the homology test results in this study have revealed that the MPB83 protein sequence of *M. bovis* is highly specific,

Table 4. Binding and amino acid residues of epitope $_{106}$KLNPDVNLVDTLN$_{118}$ to MHC class II.

Type of bond formed	Number of ties	Amino acid interaction of MPB83 protein that binds to	Binding MHC class II amino acid interactions	Bond distance (Å)	Strength
Hydrogen bonding	14	LYS-106	GLN-44	1.7	Strong
		LYS-106	GLN-41	1.7	Strong
		LYS-106	SER-83	2.2	Strong
		LYS-106	ARG-169	1.8	Strong
		ASN-108	THR-175	1.9	Strong
		PRO-109	ARG-111	1.7	Strong
		VAL-111	ASN-40	2.2	Strong
		ASN-112	ARG-113	1.8	Strong
		ASN-112	GLN-41	2.0	Strong
		ASP-110	GLN-178	2.1	Strong
		ASP-110	ARG-111	1.8	Strong
		ASP-115	CLN-37	2.1	Strong
		THR-116	TYR-10	1.7	Strong
		ASN-118	SER-110	1.9	Strong

MHC: major histocompatibility complex

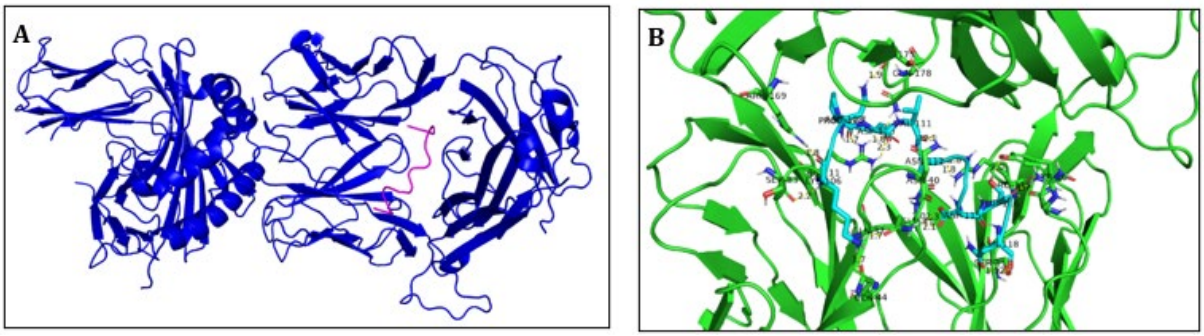

Figure 2. A. Binding interaction model number 3 illustrates the interaction of 106KLNPDVNLVDTLN118 with MHC class II. The blue model is MHC class II as the receptor, and the magenta model is the epitope $_{106}$KLNPDVNLVDTLN$_{118}$ as the ligand; B. Cartoon diagram of binding interaction model of 3 docking results of $_{106}$KLNPDVNLVDTLN$_{118}$ epitope against MHC class II (green). The drawing was made using PyMOL visualization software. The green model is MHC class II as the receptor, and the blue model is the epitope $_{106}$KLNPDVNLVDTLN$_{118}$ as the ligand. MHC: major histocompatibility complex

with a low level of conservation compared to the Ag85 protein sequence of the *M. Tuberculosis* complex, which consists of Ag85A, Ag85B, and Ag85C. This specificity suggests that the MPB83 antigen protein is a promising candidate for *M. bovis*-specific serodiagnostic.

Our results support the in vivo study by Sridhara et al., which showed that detecting IgG antibodies against MPB83 antigens is specific for optimal diagnostic accuracy. The use of MPB83 antigen to measure IgG response in *M. bovis* infected pigs has an estimated test sensitivity of 97.5%. None of the 57 control samples (uninfected pigs) were detected to have IgG and IgM antibodies to MPB83 antigen protein in the in vivo assay, indicating a specificity of 100%.[11] Percent sequence identity of the MPB83 antigen protein of *M.bovis* to the Ag85 protein sequence complex is low, less than 30%. These results indicated that the MPB83 protein is specific to *M. bovis*, so it will not cause cross-reactions with the complex Ag85 protein when used as a serodiagnostic antigen. Cross-reactions

can occur when sequences have more than 50% similarity.[22]

The results of the antigenicity test have provided evidence that the MPB83 protein is classified as an antigenic protein, meaning that the human immune system with MHC intermediaries can recognize it. This finding, supported by in vivo studies on a group of pigs infected with *M. bovis*, demonstrated that the MPB83 protein is an immunogenic protein capable of evoking an immune response by producing IgG. This potential to produce antibodies, which will later bind to the MPB83 protein to form an antigen-antibody complex for bTB diagnosis.[11] Thus, the MPB83 protein, as shown by the VaxiJen v2.0 analysis server score and in vivo studies, could potentially be used as a promising candidate biomarker for serodiagnostic bTB.

B-cell epitope prediction results from the MPB83 sample showed seven predicted epitope regions. Potential epitopes are currently more developed than whole proteins in designing antigen protein-based serodiagnostics. Lv et al. found it better to use recombinant epitopes of pGEX-Sj23-SjGCP protein antigens as diagnostic antigens rather than recombinant whole proteins in detecting the presence of *S. japonicum*. This replacement was shown to increase the sensitivity of the diagnostic biomarker to 97.8%.[23] A study also showed protein replacement with B-cell epitopes to detect antibodies in leptospirosis increased sensitivity by 5.1%-5.8%.[15]

Antigenic epitopes are expected to bind to specific parts of the formed antibodies. Therefore, the epitope predicted on MPB83 is continued with the antigenicity test using VaxiJen v2.0 and Kolaskar and Tongaonkar Antigenicity. The results of the epitope antigenicity test showed three epitopes classified as antigenic epitopes based on the VaxiJen v2.0 server. Based on analysis using Kolaskar & Tongaonkar, three antigenic epitopes were narrowed down to one epitope because the entire peptide is antigenic, namely epitope $_{106}$KLNPDVNLVDTLN$_{118}$. These results showed that epitopes with high antigenicity values could have more potential than the whole protein antigen because proteins that have several epitopes with different antigenicity values, when accumulated, can cause lower antigenicity values of the most potential epitopes.[24] This aligns with Ndille et al., who used a peptide and an epitope of Nterm-34 kDa as a biomarker of exposure to *Aedes aegypti* mosquito bites.[25] Epitopes that are part of the antigen will bind to the paratope on the antibody so that antigenic epitopes will more specifically bind to the antigen.

Based on the physicochemical character test, the epitope with the most potential for use as a serodiagnostic antigen is the $_{106}$KLNPDVNLVDTLN$_{118}$. This epitope stands out due to its stability and hydrophilic nature. A molecule is predicted to be stable if it has an instability index of less than 40, according to the Expasy ProtParam analysis server.[26] Stable epitopes, such as this one, will maintain their structure during environmental changes due to chemical and physical reactions in binding to the antibody structure.[27] Stable epitopes will affect the presentation of diagnostic biomarker epitopes and increase humoral cellular immunity. The stability of this epitope enhances epitope presentation, prolongs protein persistence for continued processing and presentation by dendritic cells, and even allows direct interaction with B-cells.[28]

The epitope $_{106}$KLNPDVNLVDTLN$_{118}$ showed hydrophilic properties when tested using the Expasy ProtParam server. These results align with Mamaghani et al., who chose hydrophilicity as the nature of the epitope designed in the SARS-CoV-2 diagnostic kit.[29] Another study also mentioned that hydrophilicity was chosen in selecting serodiagnostic antigen proteins to detect Corynebacterium pseudotuberculosis.[30] Hydrophilicity is a crucial factor in selecting serodiagnostic antigens, as it allows the epitopes to dissolve in a hypotonic environment and easily interact with the paratope region, which is part of the antibody that binds to the antigen.[29]

The molecular weight of epitope $_{106}$KLNPDVNLVDTLN$_{118}$ is 1,454.64 Da, which does not follow Nugraha's opinion, which states that in proteins, a peptide with a molecular weight of 4-5 kDa is classified as immunogenic. Nugraha also noted that some molecules weighing 2 kDa are already immunogenic.[31] The problem of molecular weight that allows epitopes to be less immunogenic can be addressed by using multiple epitopes connected using protein linkers. Previous results by Yu et al., who applied multiple epitopes connected with linker proteins, produced a molecule weighing 49 kDa.[32] A multi-epitope diagnostic design is strongly recommended to enhance sensitivity and specificity with high-density expression of antigen-antibody

interactions. Theoretically, the epitopes selected in this study are excellent candidates for designing serologic diagnosis tests. For sensitivity and specificity assessment, the designed multi-epitope should be evaluated using patient sera to validate its potential as a diagnostic biomarker of bTB in humans.

The most potential epitope, $_{106}$KLNPDVNLVDTLN$_{118}$, was then molecularly docked to the MHC class II receptor using ClusPro v.2 to assess the interaction between the two. The docking simulation was performed by blind docking, docking the ligand to the entire protein surface without understanding the target binding sites that may interact.[20] Blind docking simulation does not have a limit on the size of the search space or the number of residues, so this method can filter out as many of the best interactions as are formed.[20] The strength of the bond will be interpreted based on the binding energy value formed.[33]

The blind docking of epitope $_{106}$KLNPDVNLVDTLN$_{118}$ on the MHC class II receptor yielded 19 hydrogen bond binding interaction models, with number 3 emerging as the most potent binding interaction model based on binding energy values. We used hydrogen bonding to assess the strength of the binding interaction because the interaction between ligand and receptor at hydrogen bonding is the most stable.[34] As a serodiagnostic biomarker candidate, stability is an indispensable criterion in serodiagnostic design. The results of this study demonstrate that the bond with a binding energy value of -644.8 kcal/mol in the ClusPro score is the most robust and most stable bond between the $_{106}$KLNPDVNLVDTLN$_{118}$ epitope and the MHC class II receptor. The negative binding energy value indicates that in the hydrogen bond binding between MHC class II and the $_{106}$KLNPDVNLVDTLN$_{118}$ epitope, no energy is required from external sources but rather a bond that causes the release of energy into the environment. The strong hydrogen bond formed indicates that the $_{106}$KLNPDVNLVDTLN$_{118}$ epitope can be applied as a biomarker of bTB in humans.

In our study, we obtained 14 hydrogen bonds. The distance on the hydrogen bonds ranges from 1.7 Å to 2.2 Å, indicating strong hydrogen bonds. These results agree that the hydrogen bond distance formed from the interaction between malvidin compounds and epidermal growth factor receptor (EGFR) is less than 3Å. Interactions formed at a distance of less than 3Å indicate a strong bond.[21] The strong binding of the $_{106}$KLNPDVNLVDTLN$_{118}$ epitope to the MHC class II receptor indicates that the epitope can trigger an immune response, such as the whole antigen protein MPB83 M. bovis. It was parallel with Alam et al. that the epitope of potato virus Y antigen that can bind to MHC class II has the potential to induce the release of antibodies such as whole antigen appropriately so that it will produce antigen-antibody complexes when simulated as serodiagnostic antigens.[35] The strong binding of the $_{106}$KLNPDVNLVDTLN$_{118}$ epitope with MHC class II indicates that the epitope is related to the humoral immune response, which can be recognized by specific antibodies so that antibody-antigen binding occurs as the basis for the development of bTB serodiagnostics in humans. Therefore, the $_{106}$KLNPDVNLVDTLN$_{118}$ epitope is the most potential candidate antigen to be developed as a serodiagnostic for TB due to *M. bovis.*

Despite the limitations of our research, it is important to note that our method, while unable to definitively prove which protein chain will be effective as a serodiagnostic, has been effective in prediction and mapping to narrow the likelihood of bonded chains. This method has proven effective as a reference for future research in both in vivo and clinical trials. It is supported by Chen et al., who used an silico study to map amino acids that could potentially be used as a SARS-CoV2 vaccine.[17] Similarly, Kanagavel et al. also used in silico methods as an initial stage in finding epitopes that have the potential as markers in designing leptospirosis diagnostic tools.[15]

CONCLUSION

The B-cell epitope of the MPB83 protein sequence $_{106}$KLNPDVNLVDTLN$_{118}$ can potentially be a serodiagnostic antigen candidate for bTB in humans. To gain a more comprehensive understanding, further research in vivo, including the construction, expression, and purification of recombinant proteins, followed by ELISA (Enzyme-linked immunosorbent assay) and western blot analysis, is needed.

CONFLICT OF INTEREST

All authors declare that there is no conflict of

interest regarding the publication of this study.

ACKNOWLEDGEMENT

We want to thank all the parties that cooperated and assisted in contributing the information needed for this study.

AUTHOR CONTRIBUTION

All names listed in this research have contributed to our study. The first and corresponding authors are the originators of this study's concept idea and design. All authors were involved in the intellectual content, literature search, and bioinformatics studies. The first and second authors collect data acquisition and carry out data analysis. The first author, third author, and corresponding author prepare the manuscript. Manuscript editing and manuscript review are carried out jointly by all authors.

LIST OF ABBREVIATIONS

TB: Tuberculosis; MDR-TB: Multidrug-resistant tuberculosis; bTB: Bovine tuberculosis; M.bovis: Mycobacterium tuberculosis; NCBI: National Center for Biotechnology Information; GRAVY: Grand Average of Hydropathy; IEDB: Immune Epitope Database; MHC: Major Histocompatibility Complex; PCR: Polymerase Chain Reaction; ICT: Immunochromatography; PDB: Protein data bank; IgG: Immunoglobulin G; EGFR: Epidermal Growth Factor Receptor; TNF-α: tumor necrosis factor-α; IL: Interleukin; ELISA: Enzyme-linked immunosorbent assay.

REFERENCES

1. World Health Organization. 2021. Global tuberculosis report 2021. ISBN: 978-92-4-003702-1. Retrieved from: https://www.who.int/publications/i/item/9789240037021.
2. Irianti T, Kuswandi N, Yasin M, Kusumaningtyas RA. 2016. Mengenal anti-tuberkulosis. Yogyakarta: Universitas Gajah Mada; 11-14.
3. Quadri NS, Brihn A, Shah JA, Kirsch JD. Bovine tuberculosis: A re-emerging zoonotic infection. J Agromedicine. 2021;26(3):334–9. DOI:10.1080/1059924X.2020.1771497.
4. World Health Organization. 2017. Global tuberculosis report 2017. ISBN: 9789-24-1-56551-6 Retrieved from: https://www.who.int/publications/i/item/9789241565516
5. Devi KR, Lee LJ, Yan LT, Syafinaz A-N, Rosnah I, Chin VK. Occupational exposure and challenges in tackling *M. bovis* at human–animal interface: A narrative review. Int Arch Occup Environ Health. 2021;94(6):1147–71. DOI:10.1007/s00420-021-01677-z.
6. Collins ÁB, Floyd S, Gordon SV, More SJ. Prevalence of *Mycobacterium bovis* in milk on dairy cattle farms: An international systematic literature review and meta-analysis. Tuberculosis (Edinb). 2022;132:102166. DOI:10.1016/j.tube.2022.102166.
7. El-Sayed A, El-Shannat S, Kamel M, Castañeda-Vazquez MA, Castañeda-Vazquez H. Molecular epidemiology of *Mycobacterium bovis* in humans and cattle. Zoonoses Public Health. 2016;63(4):251–64. DOI:10.1111/zph.12242.
8. Khattak I, Mushtaq MH, Ayaz S, Ali S, Sheed A, Muhammad J, et al. Incidence and drug resistance of zoonotic *Mycobacterium bovis* infection in Peshawar, Pakistan. Adv Exp Med Biol. 2018;1057:111–26. DOI:10.1007/5584_2018_170.
9. Kementerian Kesehatan Republik Indonesia. Keputusan Menteri Kesehatan Nomor HK.01.07/MENKES/555/2019 tentang pedoman nasional pelayanan kedokteran tata laksana luka bakar, Jakarta: 2019
10. Miller MA, Gortazar C, Roos EO, Risalde MA, Johnathan-Lee A, Sridhara AA, et al. Serological reactivity to MBP83 and CFP10/ESAT-6 antigens in three suid hosts of *Mycobacterium bovis* infection. Vet Microbiol. 2019;235:285–8. DOI:10.1016/j.vetmic.2019.07.018.
11. Sridhara AA, Johnathan-Lee A, Elahi R, Risalde MA, Gortazar C, Waters WR, et al. Strong antibody responses to *Mycobacterium bovis* infection in domestic pigs and potential for reliable serodiagnostics. Vet Immunol Immunopathol. 2021;231:110161. DOI:10.1016/j.vetimm.2020.110161.
12. Stedman A, Chambers MA, Gutierrez-Merino J. Secretion and functional expression of *Mycobacterium bovis* antigens MBP70 and MBP83 in lactic acid bacteria. Tuberculosis. 2019;117:24–30. doi:10.1016/j.tube.2019.05.007.
13. Buchari. Uji serologi pada penderita tuberkulosis aktif. Jurnal Kedokteran Nanggroe Medika. 2019;2(4):18–26. https://jknamed.com/jknamed/article/view/65/57
14. Rachmayanti N, Kusmiati T. Diagnostic value of encode Tb IgG and IgM rapid test to support pulmonary tuberculosis diagnosis.

Clinical Pathology and Medical Laboratory. 2020;26(2):138–41. DOI: https://doi.org/10.24293/ijcpml.v26i2.1524
15. Kanagavel M, Shanmughapriya S, Anbarasu K, Natarajaseenivasan K. B-cell-specific peptides of *Leptospira interrogans* LigA for diagnosis of patients with acute leptospirosis. Clin Vaccine Immunol. 2014;21(3):354–9. DOI:10.1128/CVI.00456-13.
16. Potocnakova L, Bhide M, Pulzova LB. An introduction to B-cell epitope mapping and in silico epitope prediction. J Immunol Res. 2016;2016:6760830. doi:10.1155/2016/6760830.
17. Chen H-Z, Tang L-L, Yu X-L, Zhou J, Chang Y-F, Wu X. Bioinformatics analysis of epitope-based vaccine design against the novel SARS-CoV-2. Infect Dis Poverty. 2020;9(1):88. DOI:10.1186/s40249-020-00713-3.
18. Encinas M, Marfil MJ, Garbaccio S, Barandiaran S, Huertas P, Morsella C, et al. *Mycobacterium bovis* ESAT-6, CFP-10 and EspC antigens show high conservation among field isolates. Tuberculosis (Edinb). 2018;111:143–6. DOI:10.1016/j.tube.2018.06.007.
19. Farrell D, Crispell J, Gordon SV. Updated functional annotation of the *Mycobacterium bovis* AF2122/97 reference genome. Access Microbiol. 2020;2(7):acmi000129. DOI:10.1099/acmi.0.000129.
20. Hassan NM, Alhossary AA, Mu Y, Kwoh C-K. Protein-ligand blind docking using QuickVina-W with inter-process spatio-temporal integration. Sci Rep. 2017;7(1):15451. DOI:10.1038/s41598-017-15571-7.
21. Prasetiawati R, Suherman M, Permana B, Rahmawati R. Molecular docking study of anthocyanidin compounds against epidermal growth factor receptor (EGFR) as anti-lung cancer. Indonesian Journal of Pharmaceutical Science and Technology. 2021;8(1):8–20. DOI: https://doi.org/10.24198/ijpst.v8i1.29872
22. Riyanti EI, Listanto E, Ambarwati AD. Penentuan alergenisitas protein gen RB pada kentang produk rekayasa genetika berdasarkan studi bioinformatika. Jurnal AgroBiogen. 2016;11(3):81-6. DOI:10.21082/jbio.v11n3.2015.p81-86.
23. Lv C, Hong Y, Fu Z, Lu K, Cao X, Wang T, et al. Evaluation of recombinant multi-epitope proteins for diagnosis of goat schistosomiasis by enzyme-linked immunosorbent assay. Parasit Vectors. 2016;9(1):135. DOI:10.1186/s13071-016-1418-4.
24. Sanchez-Trincado JL, Gomez-Perosanz M, Reche PA. Fundamentals and methods for T- and B-cell epitope prediction. J Immunol Res. 2017;2017: 2680160. DOI:10.1155/2017/2680160.
25. Ndille EE, Dubot-Pérès A, Doucoure S, Mouchet F, Cornelie S, Sidavong B, et al. Human IgG antibody response to *Aedes aegypti* Nterm-34 kDa salivary peptide as an indicator to identify areas at high risk for dengue transmission: A retrospective study in urban settings of Vientiane city, Lao PDR. Trop Med Int Health. 2014;19(5):576–80. DOI:10.1111/tmi.12280.
26. Dutta B, Banerjee A, Chakraborty P, Bandopadhyay R. In silico studies on bacterial xylanase enzyme: Structural and functional insight. J Genet Eng Biotechnol. 2018;16(2):749–56. DOI:10.1016/j.jgeb.2018.05.003.
27. Machado Y, Freier R, Scheiblhofer S, Thalhamer T, Mayr M, Briza P, et al. Fold stability during endolysosomal acidification is a key factor for allergenicity and immunogenicity of the major birch pollen allergen. J Allergy Clin Immunol. 2016;137(5):1525–34. DOI:10.1016/j.jaci.2015.09.026.
28. Scheiblhofer S, Laimer J, Machado Y, Weiss R, Thalhamer J. Influence of protein fold stability on immunogenicity and its implications for vaccine design. Expert Rev Vaccines. 2017;16(5):479–89. DOI:10.1080/14760584.2017.1306441.
29. Mamaghani AJ, Arab-Mazar Z, Heidarzadeh S, Ranjbar MM, Molazadeh S, Rashidi S, et al. In silico design of a multi-epitope for developing sero-diagnosis detection of SARS-CoV-2 using spike glycoprotein and nucleocapsid antigens. Netw Model Anal Health Inform Bioinform. 2021;10(1):61. DOI:10.1007/s13721-021-00347-x.
30. Droppa-Almeida D, Ferreira CdS, Brito I, Borsuk S, Rodríguez JAL, Lima-Verde IB, et al. In silico screening of putative corynebacterium pseudotuberculosis antigens and serological diagnosis for caseous lymphadenitis in sheep by enzyme-linked immunosorbent assay. Vet Med Int. 2021;2021:9931731. DOI:10.1155/2021/9931731.
31. Nugraha J. Epitope mapping and it's clinical aplication. Clinical Pathology and Medical Laboratory. 2011;17(3):55–104. https://doi.

org/10.24293/ijcpml.v17i3.1168
32. Yu M, Zhu Y, Li Y, Chen Z, Sha T, Li Z, et al. Design of a novel multi-epitope vaccine against *Echinococcus granulosus* in immunoinformatics. Front Immunol. 2021;12:668492. DOI:10.3389/fimmu.2021.668492.
33. Sakinah EN, Athoillah N, Putri KYW, Armiyanti Y. In silico determination of fenthion, permethrin, and carbaryl as FFAR2 inhibitors: Type 2 diabetes mellitus pathomechanism study. JKKI: Jurnal Kedokteran dan Kesehatan Indonesia. 2023;14(3):236–47. DOI:10.20885/jkki.vol14.iss3.art3.
34. Puspitasari L, Aripin ISN, Setyaningsih EP. Molecular docking senyawa caboxamycin pada *Mycobacterium tuberculosis* polyketide synthase 13 (Pks13). Sainstech Farma: Jurnal Ilmu Kefarmasian. 2022;15(2):65–70. DOI: https://doi.org/10.37277/sfj.v15i2.1265
35. Alam A, Jawaid T, Alam P. In vitro antioxidant and anti-inflammatory activities of green cardamom essential oil and in silico molecular docking of its major bioactives. Journal of Taibah University for Science. 2021;15(1):757–68. DOI:10.1080/16583655.2021.2002550.