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Original Research Article

Analysis Interaction of Immunoglobulin G and Immunoglobulin A Against *PstS1* as a Basis Specimen Selection for *M. tuberculosis* Rapid Test Diagnostic Agent

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Abstract
Psts1 is a 38-kDa phosphate-binding periplasmatic protein which developed from the recombinant Ag38 protein in local strain of *Mycobacterium tuberculosis. Psts1* has great potential to be used as a sero-diagnosis agent to be antigen rapid test because it has several epitopes that bind to antibodies. However, it is not yet known which antibody Ag38-recombinant binds maximally between IgA and IgG.
Objective: The aim of this study is to compare the interaction between IgG and IgA on *Psts1* in silico as a basis for the selection of sero-diagnosis agents in *M. tuberculosis.*
Methods: Protein-protein docking simulations using HDOCK and PDBSum
Result: The results show that the protein *PstS1* has a higher binding sensitivity to IgG based on one of the docking models which shows a docking score of -229.70, a confident score of 0.8312 and RMSD 1.060 A. Ramachandran plot also shows that testing on this model has a protein structure that is good, with disallowed regions values of 0.5% (less than 0.8%). The results of this analysis show that the most favored regions are 90.5% with a G-factor of -0.27. The quality of the structure 3D mooring model can be said to be good because it fulfills the ideal structure requirements.
Conclusion: *Psts1 M. tuberculosis* H37Rv binds to IgG more strongly than IgA.

Keywords:
M. tuberculosis; PstS1; Immunoglobulin G; Immunoglobulin A; Protein docking
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INTRODUCTION

Diagnosis of TB in is a challenge for health workers, because it has *paucibacillary* properties, then the symptoms that appear are usually non-specific and there are many limitations to existing diagnostic tests.[1,2,3] This is due to the difficulty of diagnosis which reaches 90%.[4] The main problem with is the difficulty of identifying infected individuals due to the *paucibacillary* nature of *M. tuberculosis* bacteria and also the limited sensitivity of tests.[5] In some case, some studies suggested that antibodies have possibility play protective role in at least a proportion of otherwise healthy individuals who have a history exposure of *M. tuberculosis.*[6] Using commercial serological tests for active TB have a poor accuracy in endemic setting.[7] Sero-diagnosis is applicable in many infectious diseases such as hepatitis, AIDS etc. But no successful sero-diagnosis methods have been commercialized for TB based on *M. tuberculosis* antigen-specific IgG responses [8,9] or IgA responses. Antigen-based diagnostic tests have received special attention recently because of their high potency. One of them is the development of non-sputum-based diagnostic tests such as blood, urine, feces and saliva which have also been prioritized by the world health organization.[10]

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Table 1. IgG and IgA docking analysis with PstS1 using HDOCK

Sample	Docking score	Confident score	RMSD
Model 1 IgG	-231.57	0.8364	36.10
Model 2 IgG	-229.70	0.8312	1.06
Model 3 IgG	-226.27	0.8213	39.81
Model 1 IgA	-55.42	0.1311	94.07
Model 2 IgA	-53.38	0.1265	86.31
Model 3 IgA	-52.43	0.1244	58.06

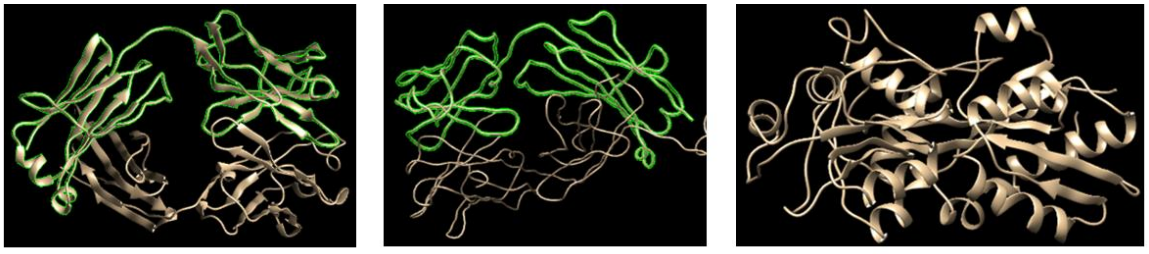
Table 2. IgG docking analysis with the PstS1 epitope using HDOCK

Sample	Model	Docking score	Confident score	RMSD
Epitope 1	1	-159.69	0.5483	47.20
	2	-153.66	0.5183	51.91
	3	-151.86	0.5093	49.49
Epitope 2	1	-120.29	0.3557	61.29
	2	-117.78	0.3443	60.76
	3	-115.75	0.3351	63.10
Epitope 3	1	-153.49	0.5174	49.94
	2	-150.64	0.5032	38.63
	3	-149.98	0.4999	39.19
Epitope 4	1	-239.45	0.8568	119.35
	2	-220.59	0.8040	116.79
	3	-220.57	0.8040	84.45

Table 3. IgA docking analysis with the PstS1 epitope using HDOCK

Sample	Model	Docking score	Confident score	RMSD
Epitope 1	1	-39.89	0.0996	90.88
	2	-39.67	0.0992	89.70
	3	-39.23	0.0984	83.67
Epitope 2	1	-30.57	0.0840	79.23
	2	-27.62	0.0796	79.52
	3	-27.16	0.0789	7079
Epitope 3	1	-34.07	0.0896	80.91
	2	-33.42	0.0885	82.88
	3	-32.14	0.0865	70.37
Epitope 4	1	-47.83	0.1147	118.04
	2	-45.99	0.1110	87.09
	3	-45.96	0.1052	105.10

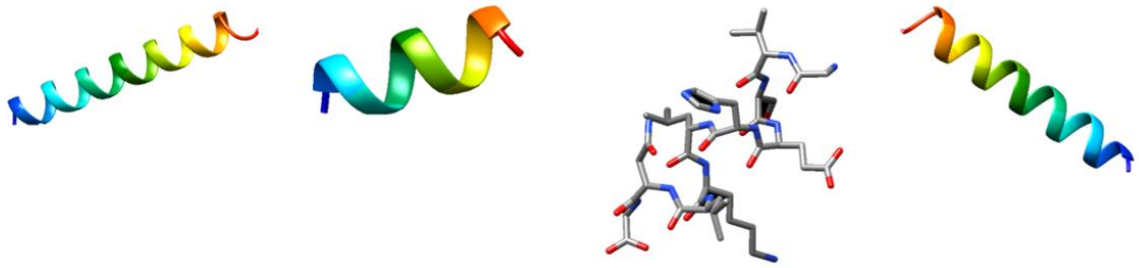
Ag38-recombinant from a local strain has been developed at the Laboratory of Biochemistry-Molecular Biology, Faculty of Medicine, Universitas Brawijaya.[11] This protein was successful in differentiating the saliva of smear-positive TB patients and healthy people using the dot blot method.[12] However, it failed to differentiate the saliva of children suspected of having TB from the saliva of healthy children.



A B C

* *Green: light chain*

Figure 1. Protein data, Immunoglobulin G (A), Immunoglobulin A (B), PstS1 protein (C)



A B C D

Figure 2. Visualization of the 4 highest predicted epitopes with Chimera 1.17.1, GSKPPSGSPETGAGAGTVATTPASSPV (A), YLSEGDMAAHK (B), GVSEHLKLNG (C), QGTIKTWDDPQIAALNPGVNLP (D)

PstS1 is an immunodominant epitope [13] which is a phosphate-binding subunit of *M. tuberculosis* (ATP-binding cassette) transporter. It is a glycosylated lipoprotein that can be found both intracellular and secreted extracellular. In this study, the Ag-38 protein or also called *PstS1* was shown to be able to trigger the appearance of IgG and IgA antibodies, both of which can recognize epitope complexes from Ag38-rec. The combination antibodies IgG and IgM had a stronger immunoreactivity to *PstS1*, this suggest that IgG is more ideal to bind *Psts1* than IgA. *PstS1* is a 38-kDa phosphate-binding periplasmatic protein, immunodominant marker for ATB [14] and plays role in M. tuberculosis immune evasion.[15] In active infection, serum antibodies against the *M. tuberculosis* phosphate transporter *PstS1* are detected.

Bioinformatics predictions of how the *PstS1* immunogenic epitope *in silico* binds to antibodies show results that may indicate further immunogenicity. It can thus be used to predict the inherent complexity of the immune presentation and epitope recognition process. Accurate prediction of the binding between the ligand and protein is critical to assisting how the target ligand interacts with the protein. The selected docking software application must be promising and provide accurate results. This requires fast and reliable computational methods using complex molecular research applications.[16] In this study a comparison will be made between the binding of immunoglobulin G and immunoglobulin A to *PstS1 in silico.* These results will represent how the prediction of bonding performance occurs such as sensitivity, specificity, accuracy of IgG and IgA, so that comparisons between the two can be known and can be used as the basis for developing a diagnostic kit saliva-based antigen antibody for *M. tuberculosis.*

MATERIALS AND METHODS

Tools and materials

The hardware used in this research were a Windows 10 pro computer with specifications Intel(R) Celeron(R) CPU N3350 @ 1.10GHz 1.10 GHz, 4.00 GB RAM, 64-bit operating system, x64-based processor. The software and website used were Protein Data Bank (PDB) https://www.rcsb.org/, National Institute of Health (NCBI) https://www.ncbi.nlm.nih.gov/, Mycobrowser https://mycobrowser.epfl.ch/, Yet Another Scientific Artificial Reality Application (Yasara) 2003, Chimera 1.17.1, Immune Epitope Database and Analysis Resourch (IEDB) with tools prediction B Cell Epitope method Bepipred Linear Epitope Prediction 2.0 (https://www.iedb.org/), HDOCK http://hdock.phys.hust.edu.cn/, PDBSum http://www.ebi.ac.uk/thornton-srv/databases/pdbsum/.

Protein and ligand data

The data used were immunoglobulin G (IgG), Immunoglobulin A (IgA) and sequence protein of *PstS1*. Protein data used a type of protein-ligand docking and the proteins used were taken from PDB with database 7DM1 for immunoglobulin G and 7k75 for immunoglobulin A. Immunoglobulin sequence modeling was analyzed using Yasara. Then the *PstS1* sequence was taken from the data in *Mycobrowser*, and an analysis of the prediction of the epitope was carried out using IEDB and visualization of the epitope with Chimera 1.17.1.

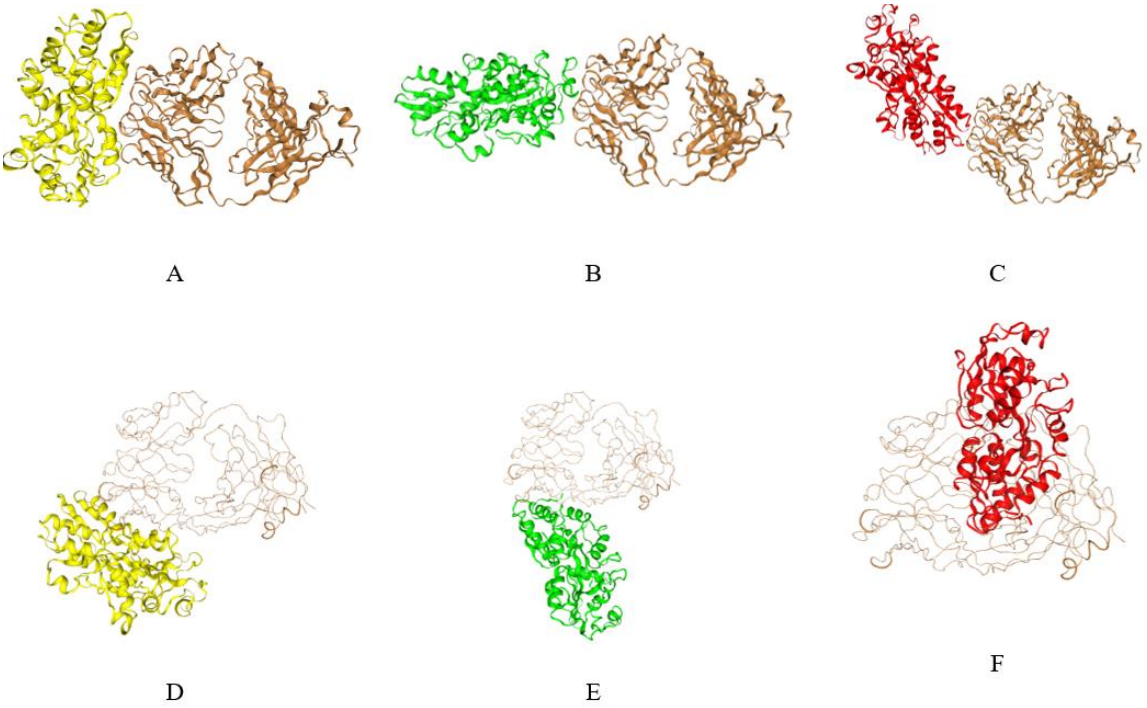


Figure 3. Modeling of IgG binding with PstS1 Model 1 (A), Model 2 (B) Model 3 (C); Modeling IgA docking with PstS1 Model 1 (D), Model 2 (E), Model 3 (F)

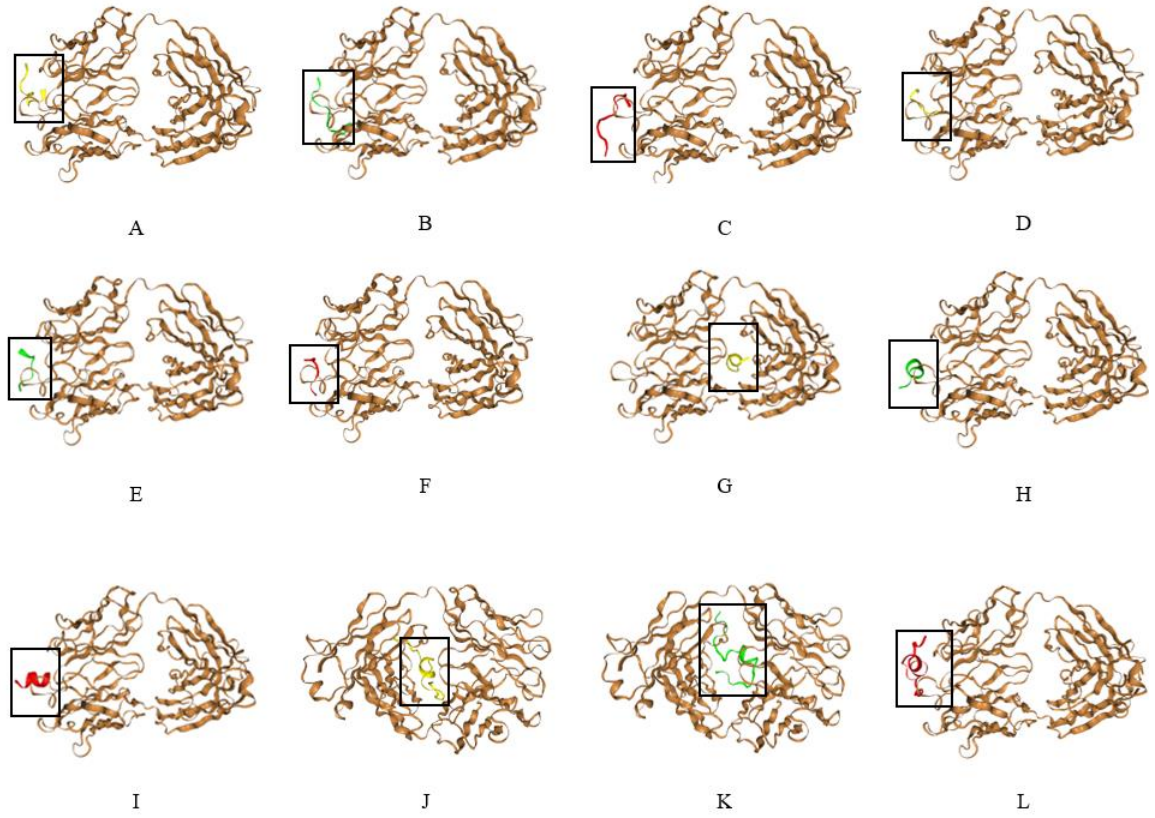


Figure 4. Visual docking of IgG with 4 highest prediction epitope of PstS1, model 1 IgG with epitope 1(A), model 2 IgG and epitope 1 (B) model 3 IgG and epitope 1 (C), model 1 IgG and epitope 2 (D), model 2 IgG and epitope 2 (E), model 3 IgG and epitope 2 (F), model 1 IgG and epitope 3 (G), model 2 IgG and epitope 3 (H), model 3 IgG and epitope 3 (I), model 1 IgG and epitope 4 (J), model 2 IgG and epitope 4 (K), model 3 IgG and epitope 4 (L)

Docking

Protein-protein docking simulations were carried out with the aim of observing, exploring, and evaluating as well as comparing the molecular interactions and affinity formed between the ligand-protein complexes obtained previously. The docking software used in this study is HDOCK and PDBSum. The success of the docking algorithm in holding the ligand binding pose can be seen from 3 parameters. The first is the value of the confidence score or the confident score which also shows the possibility of bonding 2 molecules.

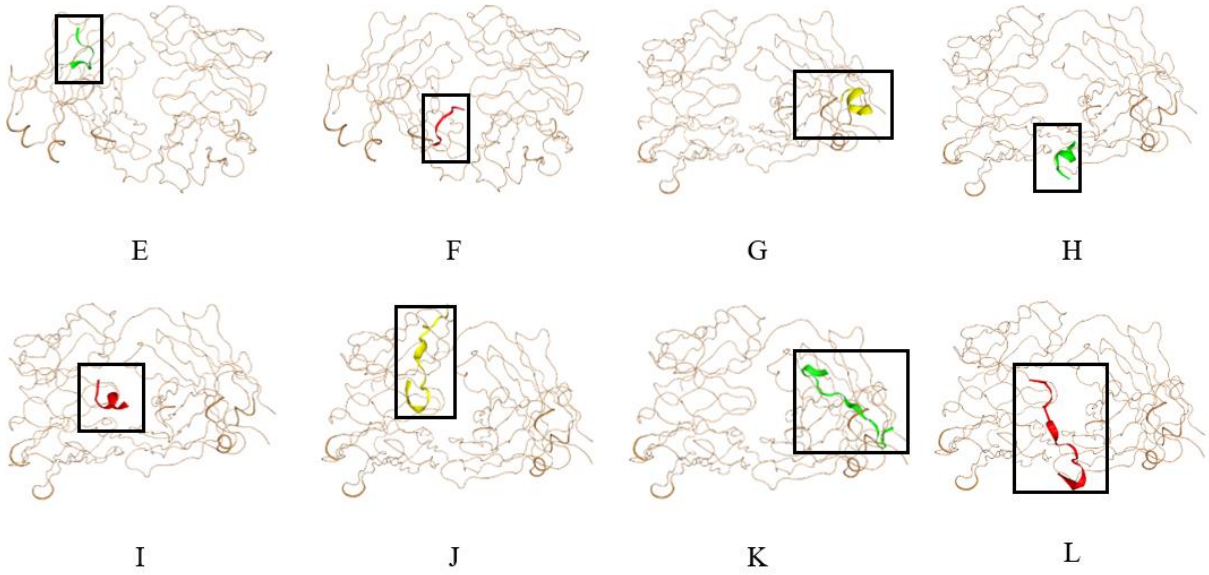
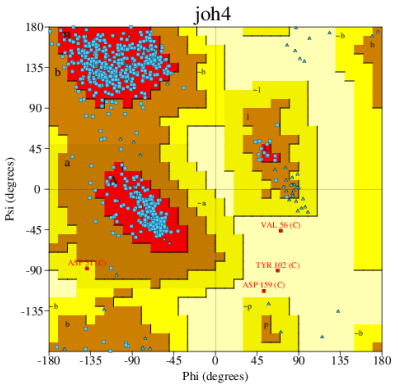


Figure 5. Visual docking of IgA with 4 highest prediction epitope of PstS1, model 1 IgA with epitope 1(A), model 2 IgA and epitope 1 (B) model 3 IgA and epitope 1 (C), model 1 IgA and epitope 2 (D), model 2 IgA and epitope 2 (E), model 3 IgA and epitope 2 (F), model 1 IgA and epitope 3 (G), model 2 IgA and epitope 3 (H), model 3 IgA and epitope 3 (I), model 1 IgA and epitope 4 (J), model 2 IgA and epitope 4 (K), model 3 IgA and epitope 4 (L)



		No. of residues	%-tage
Most favoured regions	[A,B,L]	574	90.5%
Additional allowed regions	[a,b,l,p]	56	8.8%
Generously allowed regions	[~a,~b,~l,~p]	1	0.2%
Disallowed regions	[XX]	3	0.5%*
Non-glycine and non-proline residues		634	100.0%
End-residues (excl. Gly and Pro)		4	
Glycine residues		77	
Proline residues		52	
Total number of residues		767	

Figure 6. Ramachandran plot docking on IgG with PstS1 protein

A value of 0.7 and above indicates a very high bonding ability, a value of 0.5 to 0.7 indicates the presence of moderate strength molecular bonds, and a value below 0.5 indicates a weak bond that may not even occur. The second is in the form of root-mean-square deviation (RMSD) between the positions of the heavy atoms of the ligands observed experimentally and those predicted by the algorithm. The flexibility system is a major challenge in finding the correct pose. The number of degrees of freedom involved in conformational search is a central aspect that determines search efficiency. Good performance is usually considered when the RMSD is less than.[17] Lastly is the value of the docking energy score, which is smaller than -200, the binding will be more likely to occur.

Protein Bond Conformation Analysis

Ramachandran plots were used to visualize the three-dimensional coordinates of proteins that have been determined experimentally into internal coordinates and also visualization of a function, by identifying the plots of non-glycine residues located in the dihedral corner disallowed regions. A protein structure is declared good if the number of residue plots contained in disallowed regions is less than 0.8%.[18]

RESULTS

The analysis of the mooring sites of immunoglobulin G and immunoglobulin A are shown in Figures 1A and 1B. Furthermore, the results of the *PstS1 M. tuberculosis* H37Rv sequence analysis from *Mycobrowser* which has been visualized with Chimera 1.17.1 are shown in Figure 1C. From the *PstS1* sequence, epitopes were predicted with IEDB and the 4 highest predictions were taken. The results of the prediction of the epitopes are GSKPPSGSPETGAGAGTVATTPASSPV, YLSEGDMAAHK, GVSEHLKLNG, and QGTIKTWDDPQIAALNPGVNLP. The sequence was then visualized with Chimera 1.17.1 which is presented in Figure 2. Docking of IgG, IgA with *PstS1* using the HDOCK presented in Table 1 and Figure 3. Then binding was carried out on the 4 highest predicted results of the *PstS1* epitope with IgG presented in Table 2 and Figure 4. In addition, the 4 highest predictive results for the *PstS1* epitope were also analyzed for IgA binding which is presented in Table 3 and Figure 5. Finally, the best modeling results were analyzed using the Ramachandran plot in Figure 6.

DISCUSSION

This *in silico*-based research was carried out with the aim of observing, exploring, and evaluating the structural mechanism of action of two molecular compounds between immunoglobulin, *PstS1* protein and *PstS1* epitope, as well as identifying the ability of these molecules to interact at the active binding site of the protein. The important stages of this research include the simulation of ligand-protein docking which forms a protein-ligand bond between immunoglobulin and *PstS1* protein. IgG and IgA immunoglobulin proteins that have been prepared from PDB data are prepared before removing water molecules. This preparatory stage aims to ensure the formation of molecular interactions that are able to achieve optimal stability in the active binding sites of the protein during the ligand-protein docking simulation stage and could increase the accuracy of docking. [19]

Docking begins with IgG and IgA which have been cut by light chains and heavy chains which are tethered with *PstS1* protein ligands using HDOCK. The value of the docking energy score is presented, which is smaller than -200, then the binding will be more likely to occur. From the results of the comparison between IgG and IgA the most negative docking energy score was found in model 1 on IgG binding with a value of -231.57, which means that IgG has a higher binding ability with *PstS1* than IgA. Then a confidence score is also presented which reflects the possibility of 2 molecule bonds. A value of 0.7 and above indicates a very high bonding ability, a value of 0.5 to 0.7 is for the presence of molecular bonds, and a value below 0.5 for a weak bond may not even occur. From the data that has been obtained, it is known that IgG binding has a confidence score above 0.7 in all 3 models, so IgG is considered to have the most likely bond with *PstS1*. The RMSD value shows that the second IgG model has an RMSD value below 20 or 1.060, which means that the belaying of the second model on IgG with *PstS1* has the highest probability.

In addition to docking IgG and IgA of protein *PstS1*, ligand binding was also performed on the *PstS1* epitope using HDOCK. The tethering uses the 4 selected epitopes with highest predictions from IEDB based on the *PstS1 M. tuberculosis* H37Rv sequence from *Mycobrowser*. For each epitope, 3 of the highest binding results will be taken. In ligand binding between IgG and the *PstS1* epitope, the most likely result is the binding with the predicted epitope 4 because the results of the docking score and confidence score were the best, even though the RMSD (Root mean square deviation) did not meet the standard. That is, the docking score is above -200 with a confidence score above 0.7. However, in the results of the IgA test, all docking results were not in accordance with the standard, so that docking IgA with the *PstS1* epitope was considered to have less affinity when compared to IgG.

The analysis of docking IgG and IgA against *PstS1* protein and the epitope, was found that the most probable model was IgG belay with *PstS1* protein. These results were then carried out by docking analysis with the help of PDBsum. The Ramachandran plot is used to visualize the three-dimensional coordinates of proteins that have been determined experimentally into internal coordinates. The internal coordinates consist of the dihedral angle Φ (phi) as the x-axis and the ψ (psi) angle as the y-axis of the amino acid residues of the protein structure. Mathematically, the Ramachandran plot is a visualization of a function, by identifying the plot of non-glycine residues located in dihedral regions that are disallowed (disallowed regions). A protein structure is declared good if the number of residue plots contained in disallowed regions is less than 0.8%.[20] The evaluation on IgG with *PstS1* protein has a good protein structure, if from the plot the residues found in disallowed regions are less than 0.8%, namely 0.5%. Structures with good quality are expected to have more than 90% residue in the most favored regions with G-factors above -0.5. Values below -0.5 unusual and values below -1.0 highly unusual. The results of this test show that the value of the most favored regions is 90.5% with a G-factor of -0.27. So that the structural quality of the 3D anchorage model can be said to be good because it fulfills the ideal structure requirements, namely having a residue distribution of more than 90% in the most favored regions, having little residue in the disallowed regions and having an overall G-Factors value above - 0.5. From this result, IgG have more ideal binding to *Psts1* as in study in vivo results performed exclusively in mice revealed that *PstS1* antigen is a good inducer of antigen-specific Ab responses. In addition, in mice immunized with PstS1, adjuvant LTK63 could increase the production of anti-PstS1 Ab and induce production CD^{4+}, CD^{8+} memory T cells, also amplifies secretion of IFN-γ and IL-22 and IL-17 production by effector memory cells in an Ag-unrelated manner in vitro and in vivo [21]. The other study, show that *Psts1* glycoprotein was significantly lower in IgA than IgG for detecting bovine TB, although there is no significant difference. But in that study still support feasibility using IgA and suggested an approach using tests for IgA and IgG antibodies could improve detection accuracy. [22] The limitation of this research is that it does not know the specific amino acid bonds between proteins.

CONCLUSION

The docking of IgG and IgA against *Psts1* protein showed that the most likely and ideal binding was IgG. The structural quality of the IgG and *Psts1* anchorage 3D models met the ideal anchorage requirements. This shows that IgG is more ideal as a basis sero-diagnostic for diagnostic kit saliva based antigen antibody for *M. tuberculosis.*

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