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Submission date: 19-Jan-2019 07:16PM (UTC+0700)
Submission ID: 1065991535
File name: y_with_S_aureus_and_human_spermatozoa_protein_233-239_V9N1CT.pdf (311.82K)
Word count: 3293
Character count: 18905

International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN: 0974-4290
Vol.9, No.01 pp 233-239, 2016

Molecular Modeling for Revealing Cross-Reaction Antibody with *Staphylococcus Aureus* and Human Spermatozoa Protein

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Abstract: Infertile women with non-specific vaginitis due to *Staphylococcus aureus* (*S. aureus*) will develop antibodies that cross-react with human spermatozoa, causing infertility. This study aim is to evaluate the homology of *S. aureus* and spermatozoa proteins based on sequences and structural model through computational approach. All proteins of human spermatozoa were retrieved from UNIPROT. Modeling protein was contructed based on threading modeling using I-TASSER. Sequence homology analysis was evaluated using BLASTP and structural comparison was done by Superpose v.1.0. Antigenicity and epitope mapping of homolog proteins were conducted using IEDB webservices for comparing the potential cross-reaction antibody. The result of sequence comparison showed that there are 5 homolog proteins from human spermatozoa and *S. aueus*. Based on structural anlysis, antigenicity and epitope mapping, potential candidate protein that have cross-reaction with human antibody are protein deglycase DJ-1, Sperm acrosome membrane-associated protein 4, UDP-N-acetylhexosamine pyrophosphorylase. These proteins have similiar structure and similiar position of epitope that locate on surface protein. In addition the protein have high antigenicity profile. It can be concluded that the similiar properties of sequence, structure, antigenicity and epitope from those proteins are possible if human antibody can cross-react with human spermatozoa protein after infecting by *S. aureus*. This mechanism may have role in infertile woman.

Keyword : Antigenicity, infertile woman, *S.aueus*, spermatozoa protein.

Introduction:

The incidence of infertility is quite large and varied between 7-30%[1-5]. Female factors responsible for 30-40% individually and contribute 60-70% to the spouse[1,6]. The incidence of secondary infertility is higher than primary infertility, which is due to the high incidence of STDs, medical interventions that are not hygienic especially during labor and induction of abortion[7]. Infections in developing countries had a stake of 50-80% of infertility[8].

Immune reaction in spermatozoa contribute 2-30% of the infertility[9]. Antisperm antibody (ASA) found in infertile couples at about 9 to 12.8%[10,11]. Forty-eight (48) patients with chronic salpingitis checked ASA-IgG positive 8.3% and 10.4% subpositive. ASA-IgA detected 22% positive and 28% subpositive[5]. The existence of ASA in connection with idiopathic infertility shown with barriers to fertilization process stages[12,13].

The most common cause of vulvovaginitis in women who are symptomatic are non-specific vaginitis by 40-45%[14]. *Staphylococcus aureus (S. aureus)* is a bacterium that one of the causes of vulvovaginitis[7]. Preliminary study Relations of vaginitis Non Specific with secretory immunoglobulin A cervix uteri in Women Infertile Couples in RSI Hasanah Muhammadiyah Mojokerto 2014 obtained 80% women of infertile couple have vaginitis non spesific. The dominance of bacteria isolated is *Staphylococcus sp*. by 37% (21% of *S. aureus* and *S. epidermidis* 16%) with a sensitivity to antibiotics ranged between 25-100%[15].

The female reproductive tract are unique in balancing the immune system with procreation. It is regulated by estradiol and progesterone at menstrual cycle[16,17]. Servicovagina and seminal fluid contains more IgG than IgA[16]. From the previous research result, there has been no theory that explains the relationship of non-specific vaginitis infection with *S. aureus* bacteria in women infertile couples to the particular immunological cross-reaction between antibodies of *S. aureus* protein with a spermatozoa protein. Therefore, This study aim is to evaluate the homology of *S. aureus* and spermatozoa proteins based on sequences and structural model through computational approach.

Method:

Retrieval Sequences

Spermatozoa proteins were retrived from UNIPROT (http://uniprot.org). The filter for searching is for human protein only. We selected the reviewd protein only for reliable data. There are 96 proteins from human sprematozoa proteins. The amino acid of all protein was collected automaticly in fasta format. These sequences is the main sample for comparing with *S. aureus* protein in database.

Protein modeling

The selected protein form human spermatozoa and *S. aureus* were constructed by threading modeling using I-tasser. The protein model is important for structural coparison. I-tasser can build model based on fold-recognition approach. The reliable model was evaluted based on C-score (confidence score) [18].

Sequence and structure alignment

All collected sequences from human spermatozoa were aligned with *S. aureus* protein through BLASTP NCBI. The alignment process was used local alignment to optimize the local homolog between human spermatozoa protein and *S. aureus* protein. BLAST method can detect sequence homology and give information about query cover. The BLAST process was done with *S. aures* database (TaxId: 1280). We selected the top five homolog sequences for molecular weight analysis through ProtScale Expasy tool[19]. The three dimension model from human spermatozoa and *S. aureus* were aligned for identifying the structure similarity. The superimpose analysis was done by Superpose v.1.0[20]. We evaluated RMSD of protein. The higher RMSD means the more structural different of two protein. The similiar sequence and structure can be basis information of cross-reaction antibody.

Antigenicity and epitope mapping

Antigenicity analysis is important for describing the protein can induce immune response in human body and epitope mapping analysis can be used for determining the antibody binding site of the antigenic protein. Both analysis was done through IEDB webservies. Antigenicity analyisis of 5 homolog protein were evaluated using kolaskar and tongoaskar method and structural epitope mapping was done by DISCOTOPE[21,22]. The potential cross-reaction antibody can occur if there is homolog immune properties form homolog protein.

Molecular visualization

All biomolecules were visualized using Chimera 1.8.1[23].

Result and Discussion:

Proteomics analysis can reveal the structural and fuctional of protein. The computational approach for identifiying the sequence and stuctural homolog between human spermatozoa and *S. auerus* can be describing basic information about the cross-reaction antibody in infertile woman that infected by *S.aureus*. The result showed that there are 55 of 96 human spermatozoa protein that have homology with *S. aureus* protein. 55 homolog proteins have sequence identity range between 19-45% and query cover is 5-91%. Based on this result, the protein can have similar structure and function if minimum sequence identity is 30%. The similar sequence from human spermatozoa and *S. aureus* can be proposed for cross-reaction antibody mechanism. In addition to prove that hypothetical theory, structure comparison is essential method for determining that protein have similar structure. The superimpose result showed that similarity structure based on RMSD (Table 1).

Table 1 Comparison of sequence and structural protein from human spermatozoa and *S. aureus* protein

Human Sperm Protein	*S. aureus* Protein	Homology range AA	Homology	Coverage	RMSD (Å)
Glyceraldehyde-3-phosphate dehydrogenase	Glyceraldehyde-3-phosphate dehydrogenase	4-327	44%	79%	0,314
L-lactate dehydrogenase C	Lactate Dehydrogenase	8-309	41%	91%	0,332
Protein deglycase DJ-1	Hypothetical protein1 *S. aureus*	2-166	26%	89%	4,99
Sperm acrosome membrane-associated protein 4	Hypothetical protein2 *S. aureus*	4-35	41%	25%	6,16
UDP-N-acetylhexosamine pyrophosphorylase	Uridylyltransferase	7-389	33%	79%	26,40

There are top five homolog protein from human spermatozoa protein and *S. aureus* protein. The homology is from 26 up to 44%. Glyceraldehyde-3-phosphate dehydrogenase has the highes homology (44%) and has similiar structure (0.314 A). The other proteins can be referred in table 1. The highest identity is potential have the similar structure. To prove similarity structure of the 5 proteins are then tested structural similarities. Fifth proteins have high amino acid squence homology, 3/5 (60%) of the protein has no structural homology with rmsd value of 4.99, 6.16 and 26.40. whereas 2/5 (40%) protein have structural homology because they will have rmsd value less than 2, it is 0.314 and 0.332. Evaluation of similiar structure showed the identical structure that indicated by RMSD. The lowest RMSD score means the structure of two protein are identics (Figure 1).

Structure comparison of human sperm protein with *S. aureus* protein

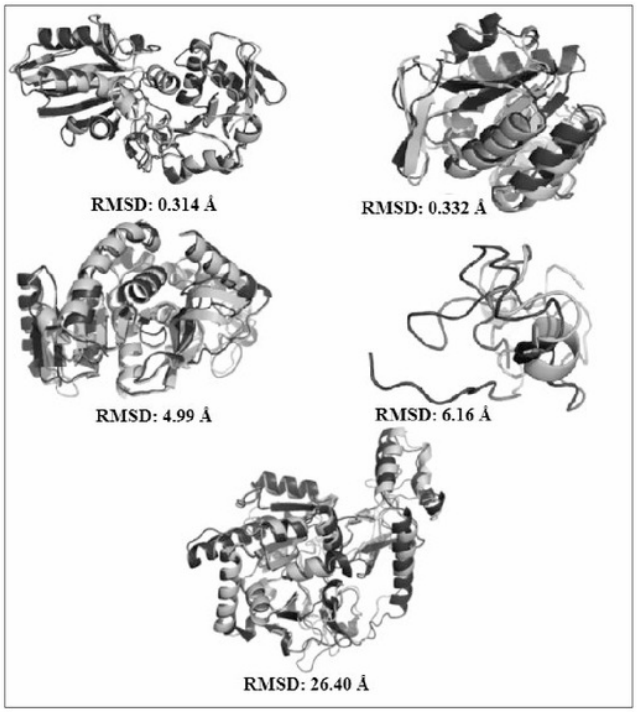

Figure 1 Evaluation of similiar structure showed the identical structure that indicated by RMSD. Structure comparison of human sperm protein (dark ribbon) with *S. aureus* protein (light ribbon)

Molecular weight (MW) is one of physical properties that can give initial information for protein identification. Some proteins have the same common name but different molecular wight (Table 2). This information can be compared to others study that found antigenis proteins from *S. aureus* have role in infertility of woman. Previous study got spermagglutination factor (SAF) is derived from 10% of *S. aureus* isolates were isolated from idiopathic infertile women in the form of a protein with MW of 70 kDa and no effect on erythrocytes[24]. Another study getting MW SAF of *S. aureus* is 57 kDa[25]. The active component of *S. aureus* is in the extracellular (in vitro studies) and in the form of a protein called sperm immobilization factor (SIF) with MW 20 kDa[26]. Previous study doing research with a mouse monoclonal antibody ASA obtain carbohydrates mediate the reaction between spermatozoa with microbes such as *S. aureus*, *Streptococcus viridans* (*S. viridans*), *E. coli*, *Salmonella typhi* (*S. typhi*) [27]. Rabbit spermatozoa membrane protein with MW 73.4 kDa, 65.8 kDa, and 45.6 kDa associated with antisperm antibodies and immunological infertility[28].

Table 2 The weight ratio of protein molecules between Human Sperm Protein and *S. aureus* Protein

No	Human Sperm Protein	MW (KDa)	*S. aureus* Protein	MW (KDa)
1	Glyceraldehyde-3-phosphate dehydrogenase	44.5	Glyceraldehyde-3-phosphate dehydrogenase	37.2
2	L-lactate dehydrogenase C	36.3	Lactate Dehydrogenase Mutant (A85r)	34.6
3	Protein deglycase DJ-1	19.8	Hypothetical protein WP_016170591.1	18.3
4	Sperm acrosome membrane-associated protein 4	13	Hypothetical protein WP_000431198.1	41.3
5	UDP-N-acetylhexosamine pyrophosphorylas	57.7	Uridylyltransferase	45.5

Based on these results we did further analysis, to determine whether the protein of *S. aureus* which has homology with human that can induce a high immune response or not (antigenicity), and the homologous region can bind to the antibody or not (epitope mapping).

Analysis antigenicity of 5 proteins of *S. aureus*, all of this show high antigetisity, so all 5 of these proteins are immunogen which means that the protein is able to evoke an immune response even to the formation of specific antibodies (Figure 2). Likewise with human spermatozoa 5 protein homologs also have high antigenicity as well, so it has the ability to generate an immune response both innate immune response and the adaptive immune response.

Antigenicity and Epitope analysis of human spermatozoa and *S. aureus* protein

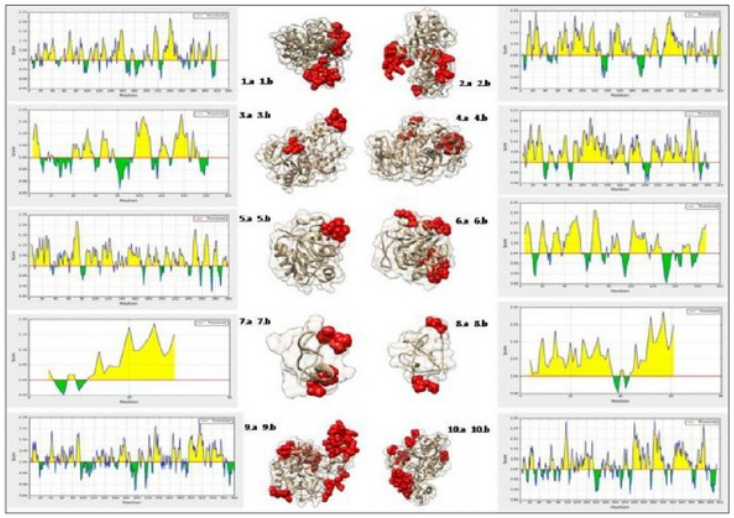

Figure 2 Antigenicity and Epitope analysis of human spermatozoa and *S. aureus* protein. Odd number (1,3,5,7,9): *Staphylococcus aureus*; Even number (2,4,6,8,10): Human Sperm; (a) antigenicity: yellow (+) green (-); (b) Epitope (red): 1. Glyceraldehyde-3-phosphate dehydrogenase; 3. Lactate Dehydrogenase; 5. Hypothetical protein1 *S.aureus*; 7. Hypothetical protein2 *S.aureus*; 9. Uridylyltransferase; 2. Glyceraldehyde-3-phosphate dehydrogenase; 4. L-lactate dehydrogenase C; 6. Protein deglycase DJ-1; 8. Sperm acrosome membrane-associated protein 4; 10. UDP-N-acetylhexosamine pyrophosphorylase

Five *S. aureus* proteins have epitope all exposed nothing is hidden. Whereas human spermatozoa from 5 proteins, only 4 human spermatozoa proteins have epitope exposed while one protein of human spermatozoa epitope is hidden namely L-lactate dehydrogenase protein C. The position of epitope in human sperm protein and *S. aureus* protein is similiar. It can be potential site when antibody bind to the protein. If the epitope is similiar, antibody can cross-react between these proteins.

Glyceraldehyde-3-phosphate dehydrogenase, testis-specific. have an important function in the setting up of glycolisis energy during the process of formation of spermatozoa and the spermatozoa function. The energy produced is used for sperm motility. The enzyme is located in the cytoplasm of spermatozoa, especially in fibrous sheath on the principle piece. While L-lactate dehydrogenase C chain, an enzyme whose function is to produce energy from glycolisis with the change in lactate into pyruvate and otherwise used in sperm motility. The enzyme is located in the cytoplasm, extracellular exosome, motile cilium and nucleus.

Protein Deglycase D1. This enzyme functions is as a positive regulator of androgen signal conduction path used during the growth and transformation of cells (spermatozoa) as modulating signaling pathways of NF-kappa-B. The location of the enzyme present in the plasma membrane, the raft membrane, extracellular exosome, also in the cytoplasm of Sertoli cells, spermatogonia, spermatids and spermatozoa. Sperm acrosome membrane-associated protein 4, the surface of the sperm membrane protein involved in the attachment of the

plasma membrane of sperm with the egg and the fusion between them during the process of fertilization. The location of this proteins is on the cell membrane of the spermatozoa, lipid anchor and secretory vesicles acrosome, and also expressed the outer acrosomal membrane matrix as well as the inside. While UDP-N-acetylhexosamine pyrophosphorylase, is an enzyme that works to change the UDP and GlcNAc-1-P into UDP-GlcNAc, and UDP and GalNAc-1-P into UDP-GalNAc. In spermatozoa the enzyme is located on the plasma membrane of the principal piece of the sperm tail, on the neck and a small portion of midpiece at the tail of spermatozoa.

Two protein that are Glyceraldehyde-3-phosphate dehydrogenase, testis-specific and L-lactate dehydrogenase C chain, lies in subselluler while three others, Protein deglycase DJ-1, Sperm acrosome membrane-associated protein 4, and UDP-N-acetylhexosamine pyrophosphorylase, expressed on the surface of a cell or cell membrane. So that only the last three proteins on spermatozoa will recognize or cross-react with anti sperm antibody formed by stimulation of *S. aureus* protein homolog.

Conclusion:

This study found that there are top five homolog protein between human spermatozoa and *S. aureus* protein. They have sequence homology from 26-44% and have structure similarity. In addition the antigenicity and epitope analysis confirmed that they possible can be recognized by the the same antibody. There are three potential candidate for immunogenic protein that induce cross-reaction antibody that are protein deglycase DJ-1, sperm acrosome membrane-associated protein 4, UDP-N-acetylhexosamine pyrophosphorylase. It can be concluded that mechanism of cross-reaction antibody is possible when the protein have high sequence and structure homology. This condition is hypothetically for infertility mechamism in *S. aureus* infected-woman.

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