Analysis of Three-Dimensional Protein Structure of CBAVD in Indonesia as a Basis for Immunotherapy to Ensure Maternal Health

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INTRODUCTION
Birth defects (congenital defects or congenital conditions) are disorders that appear at birth and can cause physical or mental disability or death. Birth defects can generally be detected in the prenatal period. However, if this is not detected in the prenatal period, it can be seen from the post-natal examination. However, there are also birth defects that are not detected until childhood and even into adulthood. Congenital Bilateral Advance Vass Deferens (CBAVD) is a birth defect characterized by azoospermia. The incidence of CBAVD is indeed very small, around 2-10%. However, if it is not handled, it will lead to problems, especially the integrity of a household. Infertility characterized by azoospermia can be corrected through surgical and non-surgical procedures. However, surgery still requires experience and further research. In addition, there are many birth defects that cannot be treated or die at an early age.

Birth defects that cause mental disorders will persist throughout life. These birth defects can have an impact on structural, functional or metabolic abnormalities. Each year an estimated 7.9 million children worldwide (approximately 6% of all births in the world) are born with serious birth defects resulting from genetic disorders or other post-conception causes such as alcohol, rubella, syphilis, deficiency Development of research on proteins at the molecular level in detail. Determine the protein structure on the laboratory research was relatively difficult because it requires sophisticated instrumentation, long research time and requires a large amount of money. The three-dimensional structure of proteins is computationally analyzed as an excellent and cost-effective alternative in analyzing protein characteristics. Prediction of three-dimensional structures is carried out by means of a homology approach, which is the best choice for determining the three-dimensional structure of proteins by searching for similar sequences in the database as a template. Research purpose of this study is identify the three-dimensional protein structure of CBAVD in Indonesia so that it can be used to obtain drugs and immunotherapy.

MATERIAL AND METHOD
DNA extraction
PBMC culture cells with 10^6 cells in 200 μl buffer B3 (a mixture of B1 (containing Guanidine hydrochloride) and B2) were incubated for 10-15 minutes at 70° C. The next step is adding 96% ethanol (Guanidine hydrochloride) and isoprotenol<25%) then centrifuged 11,000 g for 1 minute then discarded. Then for the second wash by adding 600 μl of buffer B5, centrifuged 11,000 g for 1 minute, liquid on the collecting tube was discarded, centrifuge 11,000 g for 1 minute to clean ethanol. Put in Culum to 1.5 ml eppendorf tube, add 100 μl buffer of elution that has been warmed at 70 ° C and incubated for 1 minute, then 11,000 g is centrifuged for 1 minute. The
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Figure 1: CBAVD’s first three-dimensional protein structure forms.

Figure 2: The three-dimensional protein structure of CBAVD forms the second.

Figure 3: CBAVD’s third form three-dimensional protein structure.
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Figure 4: The three-dimensional protein structure of CBAVD forms the fourth.

Figure 5: The results of the analysis of the fifth CBAVD three-dimensional protein structure.

Figure 6: The results of the analysis of the sixth CBAVD three-dimensional protein structure.
nucleotide base strands for the primer duplicated at this stage are as shown in the table below:

| Primer      | Primer nucleotide arrangement ( Primer Gene CFRT ) | CBVD protein               |
|-------------|---------------------------------------------------|----------------------------|
| FORWARD     | AGTACTCAGAC-GCTGGGCAATAACAG-CAGACCTATCTC-TA       | STQTPGQQQDQPHSTIKKLARCVD-VCIWSQPFTQEDLGPEVSR-SLQAMVPLHSLNQBEFL |
| RESERVE     | GCTCCAGGAGCTGGATCAG-GCTGGTGTGAGTCAGTCGGT-GTCGCTAGAGAGACCATGCAGAGGTC | VLRRLGYSKTLSLQKKNPVLQMY-ACGPSLRLRTWDQDLSPKLRGC-SEPWCYHTPASTSKNF |
|             | GCTGACTTGAGGATCAGGGT-GAGTCAAAAGGTCAGGGTGGT-GCAGTGGGACATGATGCTGGCAG-GTACGAGGCTGACCATGATGCTGGCAG-GAAGAATTTCTA | YSDAWANTARPYLYNNK-KISQVCCMPVPPAYSGLGMTS-VQKVEAAVSHDHGATTLQPRARIS |
|             | GCAGTGGGACATGATGCTGGCAG-GTACGAGGCTGACCATGATGCTGGCAG-GAAGAATTTCTA | KELLVAGEWVHHEGSLQPPRFGL-NNWSSQVSLRLGPGAYINTGFPFYSFCRDIWVLLYLPRSLST |
|             | GCAGTGGGACATGATGCTGGCAG-GTACGAGGCTGACCATGATGCTGGCAG-GAAGAATTTCTA | RNSCSRLRECSGTMAHSLDLDLL-DSTGHPKSASYGVDHHRHTSH-LANNFIFVEGSCICPGV |
|             | GCAGTGGGACATGATGCTGGCAG-GTACGAGGCTGACCATGATGCTGGCAG-GAAGAATTTCTA | EILARGVSWVPSWLTAAATSD-TWTLIVPQPQPEAGTTGHQHT-WLIFLLFLRGLAVQAASEY |

RESULT AND DISCUSSION

The results showed that six types of protein were translated from CBAVD nucleotides using Expasy Software. In addition, using the Swiss Prot software, it was found that 4 protein structures of CBAVD and 2 proteins did not have a three-dimensional protein structure. This is because the two proteins have no resemblance to the proteins in the protein database, so that further research is needed, especially protein isolation and protein crystallography. The results of protein modeling can vary widely and are determined by the level of homology of the target template alignment, the quality of the model, the flexibility of the structure and the software used.

In three-dimensional structural modeling, evaluation of the protein structure model is an important stage in modeling. The percentage value of the sequence identity between the target and template is a determinant of the quality of the model. The greater the percentage of identity values between the sequence and the target, the closer the model will be to the original. Based on three-dimensional structural modeling using Swiss model software, CBAVD 1 to 4 proteins have the same 9% to 22 percent. There were no similarities between CBAVD 5 and CBAVD 6 proteins.

QMEAN (Qualitative Model Energy Analysis) combines several assessment functions to estimate model quality. The two pseudo-energy assessments provided by QMEAN are the Raw Score and the Z-score. The raw score shows the pseudo-energy value calculated statistically from several parameters. The Z-score value is obtained from the experimental determination of the structure of the same size in X-ray crystallography. The smaller the pseudo-energy value in the raw score, the better, while the bigger the Z-Score the better.

In three-dimensional structural modeling, using homology modeling is a three-dimensional protein structure modeling based on the alignment of the target protein’s amino acid sequence with similar proteins whose three-dimensional structure is known protein modeling with the homology method is also faster than other methods. The ab initio method is the most difficult and complex method compared to other methods and requires a long processing time, modeling the three-dimensional structure of proteins based on energy functions can only be used in a limited manner for relatively small proteins and the resulting accuracy is also small.

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