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The Analysis Of Rbmy1 Sts Rbmi Microdeletion Gene On *Azoospermic Factor* (Azf) Region Of The Y Chromosome In Infertile Men In Palembang

Tedy Febriyanto
Jurusan Analis Kesehatan
Poltekkes Kemenkes Bengkulu
Bengkulu, Indonesia
tedyfoo@yahoo.co.id

***Abstract*−Approximately 50-80 million couples have infertility problems, and every year appeared about 2 million new infertile couples. As many as 40% of them are caused by male factors. Male infertility generally occursdue to abnormal spermatozoa. One of the causes is related to the deletion of gene onAzoospermic Factor (AZF) regionof the Y chromosome. One of the candidate genes in that region is RBMY1. This study is a descriptive study that aims to determine the molecular frequency RBMY1 gene microdeletion in AZF region of the Y chromosome uses STS RBM1 in infertile men in Palembang. The number of samples was 39 people. The analysis was done by using *Polymerase Chain Reaction* (PCR) and electrophoresis on 2% agarose gel. From this research, it was found that the frequency of microdeletions on the AZF region of the Y chromosome uses STS RBM1 of infertile men in Palembang was 7.7%. Further studies onmicrodeletion region of Azoospermic factor (AZF) of the Y chromosome in interfile menin Palembang, with other genes or by using criteria on only male factor, for example oligozoospermia, oligozoospermia extreme or cryptozoospermia were used as samples.**

Keywords−Infertile men, infertility, microdeletions, Azoospermic Factor, AZF region, Y chromosome.

I. INTRODUCTION

Approximately 50-80 million married couples by the World Health Organization (WHO) have infertility problems, and every year appeared about 2 million new infertile couples (Saraswati, 2015). Difficulty of having children was 40 % caused by male factors, and 40% caused by women factors, and 30 % caused by both factors . Therefore, the notion that women are more responsible for the difficulty in obtaining child is less precise assumptions.[1]

Causes of infertility include non-genetic factors or genetic factors. Infertility due to genetic factors that manifestedby genes level, such as mutation and microdeletion.[2]

In men, infertility generally occurs because of abnormal spermatozoa which includes concentration, motility, and morphology of spermatozoa.The causeof abnormalities in sperm concentration was related to the deletion of Azoospermic Factor (AZF) gene region in the long arm of Y chromosome.[3]

Chromosome Y of human has an important role in male fertility because they contain many genes that are essential to the process of spermatogenesis in men. Structural changes to lose or microdeletions of the region that varies on a long or short arm of the Y chromosome can cause a failure of spermatogenesis, and the genetic is mostly a cause of infertility. Spermatogenesis in the Y chromosome arrangement lies in the region of Yq11. These regions associated with the occurrence of male infertility, this region is known as Azoospermic Factor (AZF). Deletion of some parts of the region this AZF could cause disruption of spermatogenesis causing infertility. More than 30 genes on the the Y chromosome have been identifiedalthough its function on spermatogenesis has not beenknown completely.[4]

The clinical manifestations shown as a result of a deletion in AZF region are vary widely, ranging from spermatogenic starting fromformation stage of secondary spermatocytes, to syndrome Sertoli cells Sertoli syndrome type I and type II.[5] One of the candidate genes placed in the region of AZF is RBMY1.[6]

Due to the little number of researches on genes to infertile men in Indonesia, in South Sumatera Province in particular, therefore the researcher is willing to conduct a research about microdeletions on AZF microdeletion region of the Y chromosome in infertile men by using RBMY1 candidate genes of RBMI *Sequence Tagged Sites* (STS) In Palembang city.

RESEARCH METHODS

This study is a descriptive study that aims to determine the molecular distribution of the frequency of microdeletions in the region of azoospermia factor (AZF) in the Y chromosome using RBMY1 candidate genes in infertile men in Palembang. Samples of infertile men whose sperm analysis checks revealed

abnormal results. The analysis used the technique of Polymerase Chain Reaction (PCR) and electrophoresis.

DNA extraction

Semen DNA was extracted by the method of chelex-100, taken 200 mL of semen put in a 1.5 ml sterile tube, washed with PBS pH 7.4 1000 mL, then centrifuged at a speed of 5,000 rpm for 5 minutes, the supernatant was discarded. This stage is repeated 2-3 times. The supernatant was discarded, and then added 500 mL of 0.5% saponin, mixed well using a vortex. incubation for 24 hours in the refrigerator -20oC. Furthermore, vortex back for immediate melting, then centrifuged at a speed of 12,000 rpm for 10 minutes. The supernatant was discarded, Add 1000 mL of PBS, centrifuge at a speed of 5000 rpm for 10 minutes, remove the supernatant is repeated 2 times until the supernatant was clear.

The supernatant was discarded, plus 50 mL Chelex and plus 100 mL ddH2O, incubated in boiling water (using a heat-lock) for 5 minutes and then in the vortex. Centrifuged at 1000 rpm for 1 minute, incubated in boiling water for 10 minutes, centrifuged at a speed of 12000 rpm for 10 minutes. DNA would be on the supernatant (DNA containing water). Then this section was moved in a sterile tube and stored at a temperature of -200C

Oligonucleotide primer

DNA amplification process was a process to ensure the nucleotide sequences of STS test that would be used correctly. This step was an important step, because STS that was used functioningas a measuring tool. If an error occurs in the measuring instrument (STS), the results of this study is invalid.

At Table.1, it can be seen on various STS test which was employed in this study and the order of the nucleotide bases.

Table 1. Sequences of the STS test used in this study

Gen	STS	Sekuens	Results (pb)
	RBM1_a RBM1_b	5'-ATGCACTTCAG GATACGG-3' 5'-CCTCTCTCCACAAAACCAACA-3'	800

Polymerase Chain Reaction (PCR)

For each sample PCR solution made following liquids : ddH2O 9 mL, 10 mL Taq Green go, Primary Primary Forward0,5 riverse mL 0.5 mL, 5 mL DNA. Amplification by PCR methods conducted onThermal Cycle DNA with GB BIO-RAD Laboratories brand.STS program test in accordance with Table 2:

Table 2. Denaturation, annealing and extension temperature of STS

STS	Temperature
RBM1	94°C 30 detik, 58°C 30 detik, 72°C 1 menit

Electrophoresis

2 grams of agarose was weighed and put in a elemeyer glass. Added 100 ml of TAE buffer, Mixed and heated in a microwave for 1 minute 30 seconds. Then, added 5 mL of ethidium bromide, cool in the mold for 30 minutes. A total of 4 mL and 0.7 mL of loading dye leader DNA were mixed and used as a marker. PCR product of 15 mL and 50 bp DNA ladder marker inserted into agarose wells and then put into elektroforesa tool. Tool set at 100mV voltage, 400 amperes for 25 minutes then performed visualization using Gel-Doc-made equipment BIO-RAD LABORATORIES USA which is connected to a computer by using Quantity One.

Electrophoresis results were analyzed descriptively, to observe whether there is specific band on particular base pair with suitable length and placed nucleotida position as expected (See table. 1)

II. RESULTS AND DISCUSSION

A total of 39 samples were analyzed and examined met the criteria research subjects. The results of the examination of infertile men with abnormal sperm liquid cement contained in Palembang. Examination of the samples used the technique of Polymerase Chain Reaction (PCR) and electrophoresis on 2% agarose gels, visualized with UV light.

Samples found microdeletion in the AZF region ofthe Y chromosome if electrophoresisresults did not indicate a specific band, whereas if the sample used a RBMY1candidate gene with STS RBM1, microdeletion in AZF regions would not happen, in chromosome regions with the electrophoresis results showed a specific band.

Microdeletion screening methods based on PCR technology using *Sequence-Tagged Sites* (STS) specific to the Y chromosome has been published since 1992.[7] STS is a short region of the genome (200-300 bp) sequence which cannot be found elsewhere in the genome. STS-containing sequences on either end of the site is specific and unique, we can synthesize DNA primers complementary to the ends of the site.In principle, the analysis by using only one STS for the region Azoospermic factor (AZF) is sufficient to determine whether there is or there is a deletion in the region.[8]

Examples of visualization of the results of the PCR products using 50 bp DNA Ladder marker which indicate microdeletions using RBM1 STS as presented in Figure 1. STS.

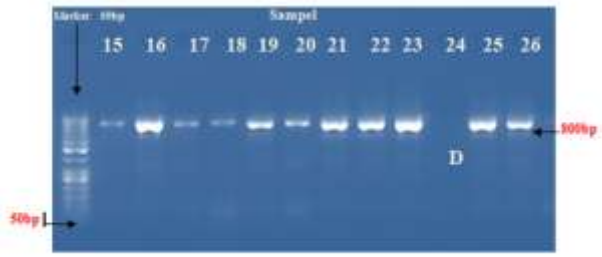

Figure 1. *Electrophoresis of PCR products with RBM1 STS in a sample of infertile men In Palembang, (D) no specific bands, this indicates the presence of microdeletions*

Examination of AZF region microdeletions of theY chromosome

After examination of the semen samples of infertile men in Palembang by using PCR and electrophoresis as a whole, there were three of 39 samples found their microdeletion on RBMY1 STS RBM1 genes in the region of Azoospermic factor (AZF). This showed the microdeletion of 7.7% in the region on AZF region of the Y chromosome of all research subjects.

Discussion

Based on the data analysis results of the microdeletion region Azoospermic factor (AZF) in the Y chromosome in infertile men using STS RBM1 above, it was found there were three of 39 samples (7.7%) who had Y chromosome microdeletions in infertile men in Palembang.The results can be compared as it is similar to some researches that have been done before. It was found 24.2% of the 99 infertile men in West Azarbaijan had microdeletion in the AZF region of Y chromosome.[9]

In another study, comparing the frequency of microdeletions in the AZF region of the Y chromosome in men Japanese and Africans showed, Out of 115 male oligozoospermia (87 Japanese and 28 Africans), there were 7 of them (6.1%) found microdeletion region AZF.[6]

Found two of 35 (5.7%) men in Indonesia were experiencing azoospermia microdeletions on Y chromosome. The deletion subregion azoospermia AZF men in Indonesia were on candidate genes on STS sY84, RBM1 STS, STS sY254 and sY255.[7] Among 226 patients with azoospermia in Slovakia, found 8 (3.35%) patients who had a microdeletion in AZF region of the Y chromosome.[10]

Data from the analysis of the Y chromosome microdeletions in 5000 infertile men from various countries have shown the prevalence of microdeletions were varied, ranging from 1% to 35% .[9]This is due to the differences in selectivity of the subject and the sample selection criteria.[6]

Microdeletions that occurred in the region of AZF candidate genes with STS RBM1 RBMY1 very specific nature of the testis, its absence in patients who are infertile same homology withoutRBM in mice that can cause infertility. [6]

Overall, based on the analysis of the above data, from AZF regionofthe Y chromosome in infertile men, the researcher found in this study that candidate genes that have been tested were likely to have roles and take a part in the process of spermatogenesis, and everyone has a tendency of each that cannot be equated between one another in terms of their relation affect to the fertility of a man.

III. CONCLUSIONS AND SUGGESTIONS

From the research on the analysis of Azoospermic factor (AZF)microdeletion region on Y chromosome of male infertility in Palembang by using RBMY1 STS RBM1 gene, obtained thatthe frequency of Azoospermic factor (AZF) microdeletion region on Y chromosome was 7.7%.

Further studies are suggested on Azoospermic factor(AZF) microdeletions region of Y chromosome in infertile men in Palembang by using other candidate genes, or using the specifications that focused on the male factor only, such as oligozoospermia,oligozoospermia extreme, or cryptozoospermia used as samples.

For couples who want to have children by IVF, it is suggested to undergo microdeletion check-up on AZF regions of Y chromosome to get information about the inheritance of genetic disorders in boys from men who are diagnosed microdeletion on Y chromosome.

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