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ADVANCES IN BIOLOGICAL SCIENCE: Respect to Biodiversity from Molecular to Ecosystem for Better Human Prosperity

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Screening of AZF Gene Deletion on Javanese and Chinese Infertile Men

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ABSTRACT

Deleted gene in the AZF (Azoospermic Factor) region in the long arm of the Y chromosome was a genetic factor presumed to be related with male infertility due to sperm abnormality. The aim of this research was to know whether sperm abnormality was determined by the locus of deletion in the AZF region and whether the location of deletion depend on the sampel ethnic. The DNA samples were extracted from periperal blood of 90 primarily infertile men that consisted of 40 Javanese men and 50 non Javanese men. Analysis of deletion based on DNA amplification (PCR) procedure used special primer of RBM and DAZ gene. The result revealed that sperm abnormality was not depend on the locus of deletion in the AZF region and the location of deletion was not depend on the sample ethnic. This research indicated that deletion in the AZF region associated with spermatogenesis arrest.

Key words : AZF region, deletion, sperm abnormality, ethnic

Introduction

Sperm abnormality was a major factor of male infertility. It was supposed that genes in the AZF region located in euchromatin region of the Y chromosome long arm (Yq11) is involved in the male infertility. This region consisted of three subregions AZFa, AZFb and AZFc (Moro *et al*., 2000; Foresta *et al*[b]., 2001; Ferlin *et al*., 2003). Most of studies on severe oligozoospermic to azoozpermic indicated there was one or more gene deletion in the AZF region (Dohle *et al*., 2002). Nevertheles, prevalence of deletion and genotype-phenotype relation between the location of deletion and sperm abnormality was not yet clear. The data showed sperm abnormality have one or two deletions in the AZF region (Osterlund *et al*., 2000; Friel *et al*., 2001), but on the other hand the different of sperm abnormality have deletion in the same locus (Seifer *et al*., 1999; Hoffer *et al*., 1999; Tse *et al*., 2000; Maurer *et a*l., 2001).

Its was suggested that the differences was due to differences in sample and locus selected for analytical purpose (Simoni *et al*., 1999). Among the proposed gene candidates, RBM (RNA Binding Motif, in the AZF b) and DAZ (Deleted in Azoospermic, in the AZF c) were two genes that always deleted in the infertile men (Foresta *et al*[a]., 2001). So far, sample

selection only based on the criterion of sperm condition and has not yet included ethnic as one of the factor.

Indonesia, one of the countries which have multiethnic population, should screen the AZF deletion on the infertile men. Besides beneficial for patients, because the deleted gene can be inherited to son, it can help the phisycians to make the right diagnosis. The aim of this research was to investigate the relation between sperm abnormality and the location of deletion in the AZF region and whether the deletion was determined by the ethnic.

Matterials and Method

Patients

This research examined 91 primarily infertile men that consisted of 45 azoospermic, 46 oligozoospermic to severe oligozoospermic (oligo-severe oligo) and 10 normospermic men (sperm concentration ≥ 20 mill / ml)as a control group. Sample ethnis consisted of 41 Javanese and 50 non Javanese (Chinese, foreign-born, Indonesian but not Javanese). The ethnic is determined to be person who married within the same ethnic over three generation. The criterion of sample selection are sperm from males who have the following condition :

1. inability to conceive after two years of unprotected intercourse
2. Oligozoospermic (sperm concentration < 20 mill /ml ejaculate), severe Oligozoospermic (sperm concentration < 5 mill /ml ejaculate) or azoospermic (no sperm in ajaculate) (WHO, 1992)

DNA extraction

DNA was extracted from pheriperal blood used the procedure of The Wizzard Genomic DNA Purification Kit. The purity and concentration of the DNA were measured by spectrophotometer UV.

Deletion Analysis

1. Amplification of the DNA with the primer pairs that specific for STS (Sequence-tagged Sites) RBM and DAZ by PCR (Polymerase Chain Reaction) method, used Thermal Cycler Perkin Elmer 9700. Amplification condition for primer RBM was pre-heat 95 º C 5 minutes, denaturation 95 º C, annealing 51 º C, elongati 72 º C each of 45 second 33 cycles and extention 72 º C 10 minutes. Amplification condition for primer DAZ was pre-heat 95 º C 5 minutes, denaturation 95 º C , annealing 61 º C, elongation 72 º C each of 45 second 33 cycles and extention 72 º C 10 minutes.
2. Visualisation of PCR result by electroforesis with 2 % agarose gel in TBE in the electroforesis chamber. Then, staining with ethidium bromide and visualisation by transiluminator.

3. Determination of deletion : DNA used to deletion test for STS RBM and DAZ were DNA that can amplified fragmen of the SRY gene (Sex-determining Region, Y chromosome) (472 bp), as a control for testis-determining factor.These DNA that can amplified the expexted size for STS RBM (550 bp) and DAZ (400 bp) indicated that the sample have DNA sequence for those genes. On the other hand, if the DNA can not amplified after three successive PCR reaction, it was a deletion.

Data analysis

Data analysed with Chi-Square (X^2) used SPSS program 11.0 Version.

Results

Deleted samples that was performed on the visualisation of PCR product were sample number 6, 22 and 23 for RBM gene and number 8, 9, 35 and 36 for DAZ gene (Figure 1).

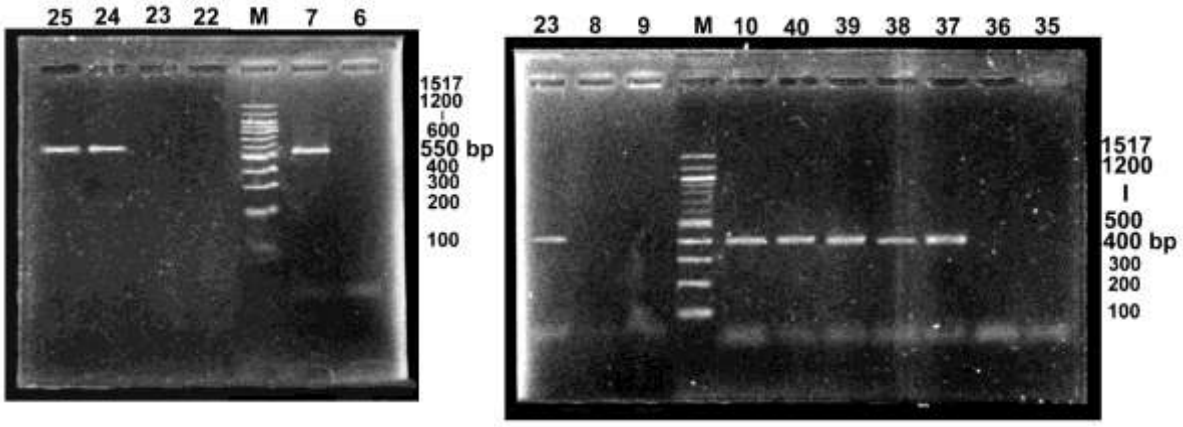
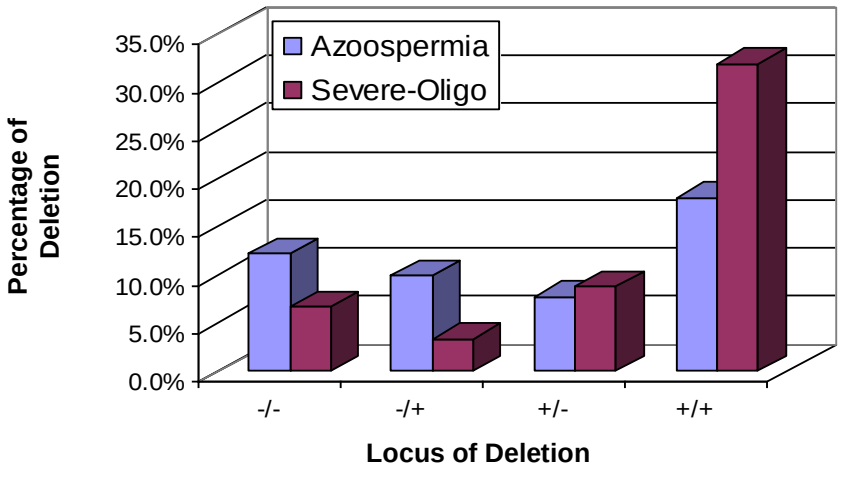


Figure 1. Visualisation of PCR product for RBM gene, 550 bp (Left) and DAZ gene, 400 bp (Rigt)

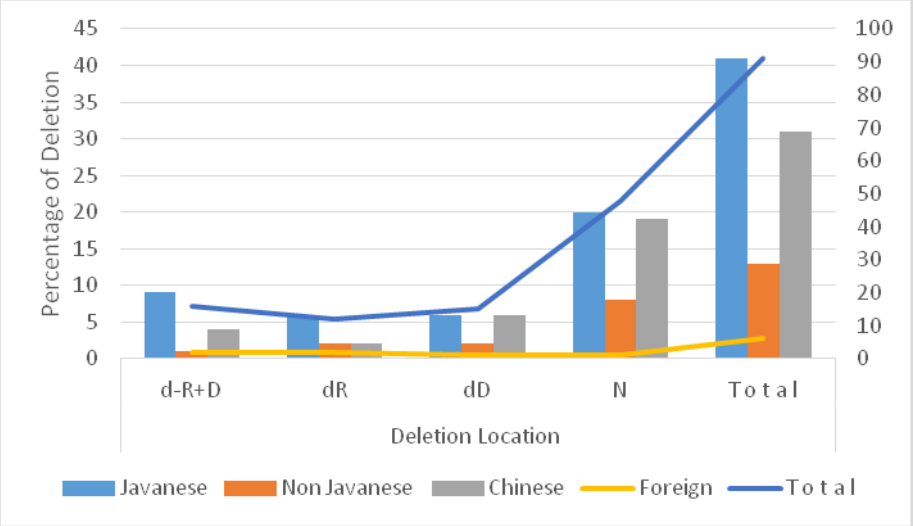
Based on the sperm abnormality, azoospermic group performed higher prevalence (29,7 %) than oligo-severe oligozoospermic (19,7 %). The highest prevalence of deletion located in the both locus RBM+DAZ (12,1%) for azoospermic group but in the DAZ locus (8,8 %) for olio-severe oligozoospermic group. (Figure 2)



-/- : RBM and DAZ -/+ : RBM
+/- : DAZ +/+ : normal (no deletion)

Figure 2. Prevalence of Subregion AZF Deletion in Azoospermic and Severe-Oligozoospermic Samples

For sample ethnic, Javanese group have the highest prevalence of deletion (23,1 %) than the other ethnics (Chinese 13,2 %; Foreign-born 4,4 % and Indonesian non Javanese 5,5 %). The highest prevalence of deletion located in the both locus RBM+DAZ (9,9 %) for Javanese group, but in the DAZ locus (6,6 %) for Chinese group while other ethnics have balance prevalence of deletion in all locus (figure 3)



-/- : RBM and DAZ -/+ : RBM
+/- : DAZ +/+ : normal (no deletion)

Figure 3. Prevalence of Subregion AZF Deletion between The ethnics

Statistical analysis indicated that deletion in the AZF region for each of sperm abnormality group was not significant ($X^2 = 7.102$ $P = 0.069$). Location of deletion in the AZF region for the sample ethnics was not significant too ($X^2 = 7.899$ $P = 0.544$).

Discussion

Azoospermic group have higher prevalence of deletion in the subregion AZF than oligozoospermic-severe oligozoospermic group. It was indicated that deletion in this subregion not only caused lost of most of the spermatogenic cells but also not any produced spermatogenic cells. For the location of deletion, DAZ locus located in the AZFc was a locus that always

deleted, not only in the azoospermic group but also in the oligozoospermic-severe oligozoospermic group. This is rather different from the former research, this research showed that deletion in both locus RBM+DAZ have the highest prevalence in azoospermic group. It's indicated that larger deletion (more than one locus) caused more severe sperm abnormality.

For Javanese ethnic, the highest prevalence of deletion located in both locus (RBM+DAZ), while for Chinese ethnic located in one locus DAZ . It was not meant that certain ethnic have a spesific deletion locus, but it was caused by the unbalance composition of sample. Javanese group have more azoospermic than Chinese or other ethnics. Because of azoospermic group have the highest deletion in both locus, Javanese group have the same cases.

The result of this research revealed that sperm abnormality can not be determined from the locus deleted in the AZF region. Deletion located in one or two locus can performed the same sperm abnormality, oligozoospermic to azoopsermic. Genotype-phenotype relationship can not be determined only based on the result of sperm analysis. It could be more clearly if there was a histology data of testis. Nevertheles, this research indicated there was a association between spermatogenesis arrest and deletion in spite of it can not be predicted that deletion located in certain locus will caused spesific sperm abnormality.

Deletion in the AZF region was not depend on the sample ethinc. All ethnic groups in this research have deletion located in the same locus in the AZF region. It was supported with the homology result of the sequensing from PCR product for gene RBM and DAZ from Javanese and Chinese sample (data was not be showed) used genetik Mac program. It showed that there was not difference between both ethnics. Recent study indicated that possibility of deletion in the AZF region depend on the Y chromosome structure such as changes in repeat sequence (Paracchini et al., 2000; Quintana-Murci et al., 2001. Indeed, AZF region has most of repeat sequences (vogt in Jansen & Martinez, 1999).

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