RINGKASAN

Delesi Region AZF (Azoospermic Factor) dalam Kromosom Y
Pria Infertil Berdasarkan Etnis di Indonesia

Evi Hanizar

Penyebab utama infertilitas pria adalah abnormalitas spermatozoa yang meliputi konsentrasi, motilitas, dan morfologi spermatozoa. Abnormalitas konsentrasi spermatozoa yang termasuk kelompok azoospermia (tidak ada spermatozoa dalam ejakulat) dan *severe* oligozoospermia (jumlah spermatozoa < 5 juta/ ml ejakulat) idiopatik diduga berhubungan dengan delesi gen region AZF dalam lengan panjang kromosom Y (Yq). Region tersebut terbagi atas subregion AZFa, AZFb dan AZFc. Berbagai hasil kajian genetik pada pria infertil dengan konsentrasi spermatozoa abnormal belum menunjukkan keseragaman hubungan genotip – fenotip tentang lokasi delesi dengan abnormalitas konsentrasi spermatozoa. Hal ini diduga disebabkan oleh perbedaan dalam hal penentuan lokus DNA, metode, kriteria pemilihan sampel serta etnis sampel. Di Indonesia, masalah infertilitas pria selama ini hanya dikaji secara klinis dan laboratories, belum ada laporan tentang kajian genetik padahal Indonesia terdiri dari multietnis.

Penelitian ini bertujuan untuk mendapatkan prevalensi delesi region AZF pada pria infertil dengan konsentrasi spermatozoa abnormal, mengetahui peran delesi terhadap berbagai abnormalitas kuantitas dan kualitas spermatozoa serta mengetahui pengaruh etnis terhadap terjadinya delesi dalam region AZF.

Sampel penelitian terdiri dari 100 pria infertil primer yang diseleksi berdasarkan hasil analisis semen, sedangkan etnis sampel ditentukan dari garis perkawinan yang mana tiga generasi diatas sampel harus mengadakan perkawinan dengan etnis yang sama dengan sampel. Analsis delesi menggunakan DNA yang diekstraksi dari darah perifer dengan menggunakan kit Wizzard Genomic DNA Purification. Selanjutnya DNA diamplifikasi melalui metode PCR (Polymerase Chain Reaction) menggunakan primer untuk STS (Sequence-tagged Sites) RBM (AZFb) dan DAZ (AZFc). Visualisasi hasil PCR menggunakan gel agarose 2 % dan *staining* dalam etidhium bromide.

Sampel yang digunakan untuk analisis delesi STS-RBM dan DAZ adalah sampel yang mempunyai gen penentu faktor testis SRY (Sex-determining Region, Y chromosome), yang sebelumnya ditunjukkan melalui amplifikasi positip terhadap primer STS-SRY; memperlihatkan pita DNA 472 bp. Sampel dikatakan delesi untuk gen RBM dan DAZ apabila visualisasi hasil PCR tidak menunjukkan pita DNA untuk STS-RBM 550 bp dan DAZ 400 bp setelah dilakukan tiga kali PCR berturut-turut. Data dianalisis menggunakan analisis statistik *Chi-Square* (X^2), dan untuk mengetahui adanya gen RBM dan DAZ, hasil PCR untuk gen tersebut

disekuensing serta dikonfirmasi dengan sekuen dari NCBI (National Center of Biotechnology Information).

Hasil penelitian ini menunjukkan bahwa prevalensi delesi pada kelompok azoospermia (29,7 %) lebih tinggi dibanding kelompok oligozoospermia hingga *severe* oligozoospermia (oligo-*severe* oligozoospermia) (18,7 %). Delesi yang melibatkan dua STS (RBM+DAZ) paling banyak terjadi pada kelompok azoospermia, sedangkan delesi pada satu STS (DAZ) paling banyak pada kelompok oligo-*severe* oligozoospermia. Hal ini berarti delesi yang melibatkan lebih dari satu STS mengakibatkan abnormalitas konsentrasi spermatozoa yang lebih parah. Keadaan ini didukung dengan hasil analisis statistik yang menunjukkan perbedaan yang signifikan ($p < 0,05$) tentang delesi antara kelompok azoospermia dan oligoastenoteratozoospermia (OAT = mereka yang mempunyai jumlah spermatozoa < 20 juta/ ml, motilitas normal < 50 % dan morfologi normal < 30 %).

Analisis delesi terhadap abnormalitas kuantitas dan kualitas spermatozoa yang meliputi konsentrasi, motilitas dan morfologi spermatozoa tidak menunjukkan perbedaan yang signifikan ($p > 0.05$). Region AZF mengandung gen-gen yang berperan dalam proses perkembangan spermatozoa, fungsi tersebut dapat diekspresikan pada tahap pembentukan spermatogonia, spermatosit atau spermatid. Hingga kini telah dikenal lebih dari 300 STS yang berhubungan dengan fungsi tersebut, sehingga untuk menghasilkan spermatozoa yang normal tidak hanya ditentukan oleh kedua gen RBM dan DAZ tetapi memerlukan keterlibatan gen lain.

Terjadinya delesi region AZF ternyata tidak dipengaruhi oleh etnis ($p > 0,05$). Masing-masing kelompok etnis (Jawa, non Jawa, Cina dan asing) mengalami kemungkinan yang sama untuk terjadinya delesi. Namun demikian etnis Jawa paling banyak mengalami delesi yang melibatkan dua STS yaitu RBM dan DAZ (9,9 %) sedangkan etnis Cina paling banyak mengalami delesi pada satu STS (DAZ). Analisis delesi terhadap karakter semen (pH dan volume), kadar hormon FSH serta volume testis juga tidak menunjukkan perbedaan yang signifikan ($p > 0,05$).

Hasil penelitian ini menunjukkan perlunya pemeriksaan genetik bagi pria infertil azoospermia atau *severe* oligozoospermia idiopatik. Selain itu perlu diadakan kajian delesi region AZF untuk STS lain dengan variabel spermatozoa yang lain misalnya vitalitas atau kapasitasi spermatozoa.

SUMMARY

Deletion of The AZF (Azoospermic Factor) Region in The Infertile Men Y Chromosome Based on the Ethnic In Indonesia

Evi Hanizar

Sperm abnormality that was involved count, motility and morphology of the sperm were the major factor of male infertility. Abnormality of the sperm count such as idiopathic azoospermic and severe oligozoospermic was presumed to be related with gene deletion of the AZF region on the long arm of the Y chromosome. This region consisted of subregion AZFa, AZFb and AZFc. Numerous of the genetic studies on the infertile men with abnormality of the sperm count have not indicated the same pattern about the prevalence of deletion and genotype – phenotype association between the location of deletion and abnormality of the sperm count. It was caused by the differences of selection of the DNA locus, method, criterion of the sample selection and sample ethnic. In Indonesia, male infertility cases have been studied in clinical and laboratory, and has not been reported about the genetic study yet.

The aim of this research was to get the prevalence of deletion of the AZF region in the infertile men with sperm abnormality, to study about the effect of deletion on the abnormalities of sperm in quantity and quality and also to study the effect of the ethnic on the chance of deletion.

Sampels consisted of 100 primarily infertile men that were selected based on the sperm analysis. The ethnic of the sample was determined from the persons who had married within the same ethnic over three generation. Deletion analysis using DNA extracted from peripheral blood by means of Wizzard Genomic DNA Purification Kit and then DNA was amplified by PCR (Polymerase Chain Reaction) machine using the primer of STS (Sequence-tagged sites) RBM (AZFb) and DAZ (AZFc). The PCR products were visualized by 2 % agarose gell and stained with etidhium bromide.

Samples used for deletion analysis of STS-RBM and DAZ were those thaat had SRY (Sex-determining Region, Y chromosome) gene, a testis-determining factor, that was perfomed by positive amplification (present of DNA band) for the primer of STS-SRY (472 bp) previously. Sampels were assessed to be deleted for RBM and DAZ genes if visualization of the PCR products unable to perform a DNA band for STS-RBM (550 bp) and DAZ (400 bp) after three successive PCR reactions. The Chi-Square (X^2) is used to analyze the data, and the presence of RBM and DAZ genes were assessed by the DNA sequences from PCR products and confirmed with the sequences from NCBI (National Center of Biotechnology Information).

The result showed that azoospermic group had a higher prevalence of deletion (29,7 %) than either oligozoospermic (oligo-severe oligozoospermic (18,7 %). Deletion involved two STS (RBM+DAZ) was the

highest in azoospermic group but the highest deletion in oligo-severe oligozoospermic was that of one STS deletion (DAZ). It indicated that deletion involved more than one gene caused the more severe abnormality of the sperm count. It was showed by the result of statistical analysis that there was a significant difference ($p < 0,05$) between deletion in the azoospermic and oligoasthenoteratozoospermic group (OAT= the men with sperm count < 20 million / ml ejaculate, normal motility of the sperm < 50 % and normal morphology of the sperm < 30 %) group.

Deletion analysis on the abnormality of sperm quantity and quality that involved count, motility and morphology of the sperm, didn't show any significant defferent ($p > 0,05$). AZF region consisted of genes that functioned in spermatogenesis. It could be performed in various steps of the spermatogonia, spermatocyt and spermatid. There was more than 300 STS that was related with spermatogenesis, therefore the normal sperm can not only be determined by RBM and DAZ genes but it needed other gene .

Deletion in the AZF region also was not determined by the ethnic ($p > 0,05$). Each of the ethnic groups (Javanese, non Javanese/Indonesia, Chinese and others) had the same condition of deletion. Nevertheles, Javanese have the highest deletion that located in two STS (RBM+DAZ, 9,9 %) but Chinese just in one STS (DAZ). Analysis of deletion on the semen character (pH and volume), testis volume, and the level of FSH hormone didn't show significant difference ($p > 0,05$).

Based on the result of this study, it's necessary to conduct a genetic screening on azoospermic or severe oligozoospermic idiopathic infertile men. It's still needed to do other genetic studies on the AZF region with other STS and sperm character such as vitality and capacitation of the sperm.

ABSTRACT

Deletion of The AZF (Azoospermic Factor) Region in The Infertile Men Y Chromosome Based on the Ethnic In Indonesia

Evi Hanizar

Sperm abnormality is the major factor of male infertility. Idiopathic azoospermic and severe oligozoospermic are abnormality related to low number of sperm which is presumed to be related with gene deletion of the AZF region on the long arm of the Y chromosome. This region consisted of subregion AZFa, AZFb and AZFc.

The aim of this research is to study of the effect of deletion on the sperm abnormality and whether the location of deletion depend on the ethnic.

DNA extracted from peripheral blood of 100 selected infertile men based on the evaluation of semen. DNA was amplified by means of PCR (Polymerase Chain Reaction) machine using the primer of STS (Sequence-tagged sites) RBM (AZFb) and DAZ (AZFc). The PCR products are visualized by 2 % agarose gell and stained with etidhium bromide.

The result showed that azoospermic group have higher prevalence of deletion (29,7 %) than either oligozoospermic (oligo-severe oligozoospermic) (18,7 %). Deletion involved two STS (RBM+DAZ) are the highest in azoospermic group but the highest deletion in oligo-severe oligozoospermic is one STS deletion (DAZ). All of the deletion analysis on the sperm abnormality that involved count, motility and morphology of the sperm, don't show any significant defferent ($p > 0,05$). The ethnic is also did not show any difference on the deletion pattern of the genes. significant different of deletion ($p > 0,05$).

STS-RBM and DAZ carrying genes seem to have any relation with the severity of sperm abnormality. It's becaued there are 300 STS in the AZF region that consist of genes related with spermatogenesis. Therefore, it's must be other gene needed to product the normal sperm. Extensive deletion will performed more severe sperm abnormality up to absence of the sperm. Deleted genes in the STS RBM and DAZ are'nt determined by the ethnic of samples, it can be deleted on the all of ethnic groups.

Key words : AZF (Azoospermic Factor) region, deletion, male infertility, azoospermic, *severe* oligozoospermic, spermatogenesis.