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Submission date: 24-Feb-2021 10:48PM (UTC-0500)
Submission ID: 1517603859
File name: Asia_Life_Science.pdf (197.71K)
Word count: 3644
Character count: 19306

ISSN: 01173375
Volume 10, Issue 04, September, 2020

DAZ Gene Deletion Occurs in Indonesian Infertile Men with Abnormal Sperm Regardless of Ethnic Background

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ABSTRACT— The purpose of this study is analyzing the DAZ gene deletion in infertile men oligozoospermia to azoospermia on Javanese and non-Javanese ethnics in Indonesia and whether the occurrence of deletion is affected by ethnic. A hundred sample from infertile men consisting of 45 Javanese and 55 non-Javanese ethnics were used. The sperm were selected and categorized based on the results of semen analysis which includes volume and pH, concentration, motility, and morphology. The genom DNA was extracted from peripheral blood and analyzed for DAZ gene deletion. Analysis of deletion for each ethnic used descriptive approach while the effect of deletion on the ethnic used Chi-square statistic. The results showed that 5 samples (11%) had DAZ gene deletion in Javanese and 7 samples (13%) in non-Javanese ethnic. Based on the category of sperm abnormalities, 5 samples with DAZ gene deletion in Javanese ethnic consisted of 4 and 1 samples with azoospermia and severe oligospermia group, respectively. In non-Javanese ethnic, 3 samples are from azoospermia group and 4 samples are from severe oligozoospermia group. None deletion occurred in the oligozoospermia group from both ethnics. Both ethnics had 50% asthenozoospermia, meanwhile, 16 samples (62%) from the Javanese and 12 samples (43%) from the non-Javanese had teratozoospermia. It indicated that the DAZ gene deletion affected spermatogenesis to produce the quantity and quality of sperm. Also, the deletion occurrence was randomly occured and regardless the ethnic background.

KEYWORDS: DAZ, ethnic, Javanese, deletion.

1. INTRODUCTION

Infertility in Indonesia is still being a problem; even for particular ethnic, getting descent with the male gender is a family demands. The general public assumes that infertility in a family is caused by the wife, however, approximately 40-50% of factors causing infertility is caused by the men [1], [2]. It means that both genders contribute equally to the causes of infertility. This knowledge needs to be understood by the public so it can reduce discrimination from the female side that happened so far. Different from female infertility that was caused by many factors, the main cause of male infertility is sperm abnormalities with the category of severe oligozoospermia (sperm count <5 million per ml of semen) to azoospermia (no sperm in the semen). Many research of infertile men showed the sperm abnormalities associated with gene deletion in the long arm of chromosome Y (Yq11), in a region known as AZF (Azoospermia Factor). The region is divided into three subregions, namely subregion AZFa, AZFb, and AZFc. The genes in this subregion are believed to have function in controlling the spermatogenesis process so that if they get a deletion, it will produce sperm abnormalities. From the third subregions, the genes in the subregion AZFc, are the most frequently happened in the deletion of infertile men compared to subregion AZFa or AZFb [3–8]. One of the

most interesting genes for study in the subregion AZFc either the prevalence or the function is *DAZ* gene (Deleted in Azoospermia). This gene is found not only in humans but also in other primates like monkeys and gorillas. *DAZ* gene is a multicopy gene, transcribed in testis, encodes an RNA-binding protein and very polymorphic [9,10]. Furthermore, another research identified four *DAZ* genes that arranged in two clusters, *DAZ1* paired with the *DAZ2* and *DAZ3* paired with *DAZ4* [11]. This gene consistent deletion happened to azoospermia men though the prevalence is still varied [12]. This is reflected in some of the research results, which found that 83.4% deletion occurred in the subregion AZFc of infertile men in West Azerbaijan associated with *DAZ* gene, while it was found that the deletion of *DAZ* gene in Iranian infertile men only 3.9 % [13], [14]. The research results from another place showed 8.5% of *DAZ* gene deletion occurred in China infertile men, 8.8% occurred in infertile men of Tunisia, and 8% reported by Fernandez et al from Germany [15–17]. Indonesia, a multiethnic country, need to explore genetic research on male infertility as hundreds of infertile men who come to infertility clinics come from various ethnic groups [18]. They are trying to get offspring and do not know their infertility status. During this examination of infertile men are clinical and laboratory so that the results of genetic research will highly help doctors to do appropriate medical action. As for patients, they will gain certainty about the status of their infertility and they avoid unnecessary therapies so that they will save costs. Therefore, deletion of the gene can be passed down from father to son, then the deletion possibility of child infertility produced from a marriage can be early prevented. The purpose of this research was to know the prevalence of *DAZ* gene deletion in infertile men and whether the deletion is influenced by ethnicity.

2. Materials and Method

2.1 Patient Selection

The case-control research used samples that consisted of patients who went voluntarily to the infertility clinic and had signed informed consent. This research was approved by the Research Ethics Committee of the Research Institute, Airlangga University by Ethical Clearance No.002/PANEC/LEMLIT/2003. The criteria for the sample are as follows: 1) Primary infertile, meaning that had never managed to impregnate his partner at least two years after unprotected intercourse; 2) Samples derived from descent who held marriage with the same ethnic group for three generations. A man is called as certain ethnic if the previous three generations always held a marriage with the same ethnic; 3) Control samples were fertile men who have had their own descendants. The number of samples was 100 infertile men, and classified into two groups: 45 Javanese and 55 non-Javanese. For non-Javanese ethnic largely composed of Chinese, and some other ethnic groups such as Batak, Madurese, Bugis, Manado, and Arabic.

2.2 Semen Analysis

Sperm analysis carried out for the volume and pH semen, number, motility (movement) and morphology (shape) of the sperm, and was assessed based on the WHO standard procedure (World Health Organization 2010). Each semen parameter was evaluated separately and independently.

2.3 PCR analysis

For the preparation of PCR analysis, the genomic DNA was extracted from peripheral blood leukocytes using a DNA extraction kit (Wizard Genomic DNA Purification, Promega, USA). The purity level and the amount of DNA were measured using a UV spectrophotometer. DAZ gene deletion analysis was performed through DNA amplification using STS (Sequence Taq Site) specific for DAZ gene, i.e. sY254. Prior to DAZ gene deletion analysis, all samples were screened for SRY (Sex-Determining Region Y) gene, as a control factor testicle (Testis Determining Factor), the gene responsible for initiating the determination of male sex.

The samples with SRY positive were then analyzed for the presence of the DAZ gene deletion. DNA amplification was performing with the reaction mixture (PCR buffer, Taq polymerase, dNTPs and MgCl2) 12.5 μL, one pair of each primer 2.5 μL, DNA samples, and sterilized water until the total volume of 25 μL. The DNA derived from normal and fertile men were used as the positive control, while the negative control used DNA of normal and fertile women. Sterilized water was used as a control reagent, which is used throughout the PCR reaction components. PCR results were visualized by electrophoresis with the 1% agarose gel, stained with ethidium bromide and observed on UV transilluminator. Primer sequences for *DAZ* gene were 5'-GGGTGTTACCAGAAGGCAAA-3' and 5'-GAACCGTATCTACCA AAGCAGC-3' which produced PCR Products of 400bp. PCR condition for DAZ gene were pre-heat 95° C for 5 minutes, denaturation 95° C, 61° C Annealing, elongation 72° C respectively for a total of 33 cycles of 45 sec and extension 72° C for 10 minutes at the end of the cycle. Samples were assessed as having a deletion if the amplified DNA did not produce PCR products corresponding to the expected size after three times repetition.

2.4 Data Analysis

The data on the prevalence of DAZ gene deletion on each ethnic was analyzed descriptively. To determine whether the incidence of deletion affected by ethnicity, the data were analyzed by X2 - test using statistical R version 3.1.3, and if the P-value <0.05 rated significant.

3. Results and Discussion

Based on the analysis of sperm obtained three categories abnormalities in sperm count namely oligozoospermia (sperm count <15 million / ml of semen), severe oligozoospermia (sperm count <1 million / ml of semen) and azoospermia (no sperm in the semen). Javanese samples consisted of oligozoospermia 8 people, severe oligozoospermia 18 people and azoospermia 19 people, while for non-Javanese ethnic consisted of oligozoospermia 11 people, 17 of severe oligozoospermia and azoospermia 27 people. The interesting thing was all samples oligozoospermia and severe oligozoospermia group of both ethnic were asthenozoospermia. It meant the sperm not only have abnormal in amount but also motility. Asthenozoospermia was the category for the number of progressives (a category) sperm motility (movement) was moving fast and straight forward, no one reached 32%. Half the sperm of both ethnics did not show a progressive move, while the other half was moving progressively only 1-5%. Sperm morphology analysis showed 16 samples of Javanese ethnic and 12 samples of non-Javanese were teratozoospermia which was the number of sperm had normal morphology (head and tail) was less than 4%. Different conditions from the measurement volume results and pH semen, 78% of all samples had normal semen volume (≥ 1.5 ml) and 56% of the samples had normal pH (≥ 7.2), the rest had a pH from 6.4 to 7. Previously, we have screened SRY gene for all the samples [19]. In this report, in Figure 1, the samples were analyzed for DAZ gene deletion, some samples (91,92) showed deletion for not being able to amplify DNA primer sY 254 with a size of 400 bp (Figure 1). The number of samples experienced a deletion of each Javanese and non-Javanese ethnic are presented in Table 1.

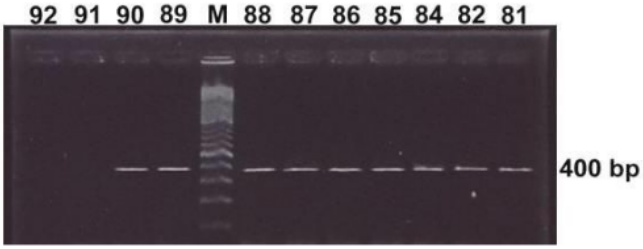

Figure 1. Visualization of PCR product for DAZ gene. M: Marker 100 bp DNA Ladder

Table 1. Number of DAZ gene deletion samples in Javanese and non-Javanese Ethnic

Results of Analysis	Ethnics		Total	Prevalence
	Javanese	Non Javanese		
Deletion	5	7	12	12 %
Normal	40	48	88	88 %
	45	55	100	

The number of samples was experienced deletion classified into Indonesian and non-Indonesian. Then 8 samples experienced deletion from Indonesia and 4 samples deletion of non-Indonesia. Based on the number of sperm abnormalities category, deletion occurred in each group oligozoospermia, severe oligozoospermia, and Azoospermia (Table 2). Statistical analysis of the deletion occurred in Javanese and non-Javanese ethnic showed that there is no effect of ethnicity on the incidence of deletion ($P>0.05$).

From the three parameters of sperm, including the number, motility, and morphology, abnormalities in sperm number (oligozoospermia, severe oligozoospermia, and azoospermia) more getting attention than the abnormal motility and morphology. The results of sperm analysis research found that all samples with oligozoospermia and severe oligozoospermia categories also include the asthenozoospermia category and half of all samples including the teratozoospermia category. They not only decrease the number of normal sperm but also decrease the quality of motility and morphology of sperm. If the research is limited to the relationship between the number of sperm motility and morphology only, not other factors involved such as deletion, hormones or antioxidants, there is no guarantee that the decrease in the number of sperm also automatically followed by a decrease in the quality of motility and morphology. Based on epidemiological data, indicated that the reduced quantity and quality of sperm related to environmental aspects such as diet and pollutants, but it is not clear how the mechanism of connection [15].

Table 2. Number of DAZ gene deletion based on sperm abnormalities group on Javanese and non-Javanese ethnic

Categories of Sperm Abnormality	Javanese		Non Javanese	
	Deletion	Normal	Deletion	Normal
Oligozoospermia		8		11
Severe oligozoospermia	1	17	4	13
Azoospermia	4	15	3	24
	5	40	7	48

Various research had shown the role of AZF gene deletion to decrease the number of sperm (oligozoospermia and severe oligozoospermia) to the absence of sperm in the semen (azoospermia) [20–23]. The deletion role of the two parameters of sperm simultaneously namely the decrease in the number and motility of sperm was found by [24], although this condition occurs only in one case. The research found the presence of a deletion in the subregion AZFc in oligoasthenozoospermia infertile men. The relationship between the decrease sperm motility with other factors other than a deletion was shown by [25] that decreased levels of lipid peroxidation and antioxidant correlated with the decrease in sperm. While the correlation between the level Follicle Stimulating Hormone with the concentration of sperm assessed by [26], while [27] found that there is a moderate correlation between parameters Sperm Chromatin Structure Assay (SCSA) with sperm motility. Much earlier, [28] had found that sperm motility is affected by mutations in mitochondrial DNA (mt DNA) of sperm infertile men. the decline in the quality of sperm

morphology seems to still require a lot of research. The data of *DAZ* gene deletion from this research demonstrate the suitability with other research before. Overall, deletion occurred only in the group of severe oligozoospermia and azoospermia. None deletion occurs in the oligozoospermia group. As mentioned above that oligozoospermia and severe oligozoospermia group also include the asthenozoospermia category. Even those who had deletion also included the teratozoospermia category. This means *DAZ* gene deletion occurs in groups of azoospermia and severe group oligoasthenoteratozoospermia (OAT). These conditions indicate that the *DAZ* gene deletion can lead not only to a decrease in sperm number but also a decrease in the quality of sperm motility and morphology. It seems different in volume and pH semen parameters, sample of *DAZ* gene deletion experience had normal volume and pH semen. Semen found in the ejaculate that is composed of fluid produced from the vas deferens, seminal vesica, prostate glands, and mucous glands especially bulbourethral glands. The results of the parts function are normal semen volume that is > 1.5 ml and semen pH between 7.2 to 7.8 which is the normal pH for the life of sperm. Meanwhile, the DAZ gene is believed to play a role in the process of spermatogenesis, especially related to the number of sperm. Thus the *DAZ* gene deletion is not associated with volume and pH semen produced. The influence of ethnicity on the *DAZ* gene deletion from the statistical analysis result indicated that the deletion of the incident is not affected by ethnicity. One of the mutation characteristics is random events, it is because no one can predict whether a certain gene will be mutated in a specific cell or a specific generation. In the case of a gene to be mutated in an individual, it is not known which individuals would have mutations and which are not. Thus, *DAZ* gene deletion is one type of gene mutation, can occur in any ethnic group and the appearance is the tendency for the deletion to occur. Mutations can be caused by various external factors such as chemicals, radiation or naturally occur due to the failure of copying DNA accurately during cell division.

4. Conclusion

This research confirms the results of the previous research that DAZ gene deletion occurs in infertile men with severe sperm abnormalities categories namely severe oligozoospermia and azoospermia. Therefore, DAZ gene deletion is not affected by ethnicity, infertile men who will undergo a medical procedure to get a decent should also do a genetic test, to confirm the status of their infertility.

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