RESEARCH ARTICLE

PRELIMINARY STUDY CYP2A6 GENE VARIATION IN MALE INFERTILE

Jihadin R Mumtaz[1,*], Zeti Harriyati[2], Chris Adhiyanto[3], Nurul Hiedayati[4], Yona Mimanda[5], Muniroh Muniroh[5]

[1]Medical Study Program; [2]Departement of Biology; [3]Departement of Biochemistry; [4]Departement of Pharmacology; [5]Departement of Clinical Pathology;

*Corresponding Author: jihadinmumtaz@gmail.com

ABSTRACT

Background: Infertility is a condition which is described by WHO as inability for having children after one year of relationship without birth control. This condition can be caused by the male and female factor. In male infertility can be internal and external factor. The external factor is free radical, chemical substances etc. Smoking can be increased free radicals that have impact on fertility man.Smoking can increase free radicals that have an impact on fertility in a man. The CYP2A6 gene is a coding gene for the xenobiotic metabolic enzyme, in this case nicotine, which can cause infertility in men. Mutations in this gene can lead to nicotine buildup, increasing nicotine effect thus increasing the risk of infertility in men. The aim of this study was to determine whether there were variations in the CYP2A6 gene in male infertile patients.

Methods: A descriptive study using DNA from infertile and fertile men to detect variations in the CYP2A6 gene.

Result: This study showed that there were variations in the CYP2A6 gene that always found in infertile patients that is at 7610, 7661, 7788, and 8040 nucleotide posititon of the CYP2A6 gene.

Coclusion: The conclusion of this study is that there are CYP2A6 variations in infertile male.

Keywords: CYP2A6, infertility, male.

INTRODUCTION

Infertility is a condition which is described by WHO as inability for having children after one year of relationship without birth control. Around 48.5 million people have had this condition caused by multiple factor that came from either male or female counterpart with the same possibility (40-50% for each side).[1,2]

In the male counterpart, infertility conditions may be caused by inability to achieve one of the normal perimeters of the semen quality and quantity together with inappropriately timed intercourse.[1] There are many factors that can cause this condition and it is caused both from inside and outside the body. One of the major outside factors is exposure to chemical substances, which in this case nicotine substance that came from cigarettes. Nicotine is a well-known addictive substance that can affect reproductive function. It takes a metabolic process to convert nicotine into its inactive form, cotinine, so that it can be flushed out of the body. The metabolic processes take place in the liver with the help from enzyme CYP2A6.[3,4]

Xenobiotics is a chemical substance found within an organism that is not naturally produced inside the organism.[5] CYP2A6 is one of P450 enzyme for xenobiotic metabolism which responsible for 70-80% metabolism of nicotine.[3] CYP2A6 as a gene had a lot of variation especially Single Nucleotide Polymorphism (SNP) which that can cause change in the enzyme effectiveness.[6] Mutation that causes reduced up to losses of function can causes nicotine build up inside the body increasing nicotine effect, in this case, in the male reproductive system that can be lead to infertile condition in male. In this preliminary study we aimed to see if there is variation of the CYP2A6 gene in infertile men.

MATERIALS AND METHODS

This research is a descriptive study, using semen samples from infertile men and fertile men. The number of samples used were 5 infertile and 5 infertile men.

DNA Preparation and Extraction

The semen samples were washed twice with PBS solution before DNA isolation. The first steps semen sample

300 µl in microtube was washed with PBS 1900 µl and centrifugation at 1900 rpm for 20 min, supernatan discarded and pellet are used. The second steps pellets obtained from steps 1 added PBS solution 900 µl then centrifuged at 1900 rpm for 20 min. After washing the semen sample was added PBS solution 300 µl and stored in -20^{0}C refrigerator for futher DNA isolation. Sperm DNA isolation using a PROMEGA Extraction Kit® for whole blood with several modifications. The isolated sample DNA is then stored -20^{0}C before analysis.

PCR and Sequencing

The primers used to analyze the CYP2A6 gene this are as follows; forward 5"-CAC CGA AGT GTT CCC TAT GCT; reverse 5"-AAA ATG GGC ATG AAC GCC C-3". The PCR cycle used is as follows: initial denaturation (95^{0}C, 3 min), followed 35 cycles of denaturation (95^{0}C, 20 second), annealing (60^{0}C, 30 seconds), extension (72^{0}C, 3 min) and finaly (72^{0}C, 5 min). The PCR product is checked by 2% agarase gel electrophoresis and confirmed that the band size was 1500 bp with a 100 bp marker.

After obtaining the PCR product, then sequencing is carried out by sending the sample to the 1st base and result are analyzed with, we used Bioedit Sequence Alignment Editor 7.2.6.1.

RESULTS

In this study, we search for variation nucleutide on reference SNP (rs) CYP2A6 gene (table 1) that is always found in the infertile male patients.

Table 1. The Commnon of Variation Nucleutide on CYP2A6 in Infetile Male Patiens

CYP2A6 Position	Position in The Chromosome	No. rs	Variation
8250	chr19:40844073	rs2431413	(A>G)
8409	chr19:40875820		T>G/C
8428	chr19:40843895	rs775855007	(G>A/G>C)

Ref: NCBI

We amplified the fragment target of CYP2A6 and produced the PCR product 1500bp which the product can be see at the figure 1.

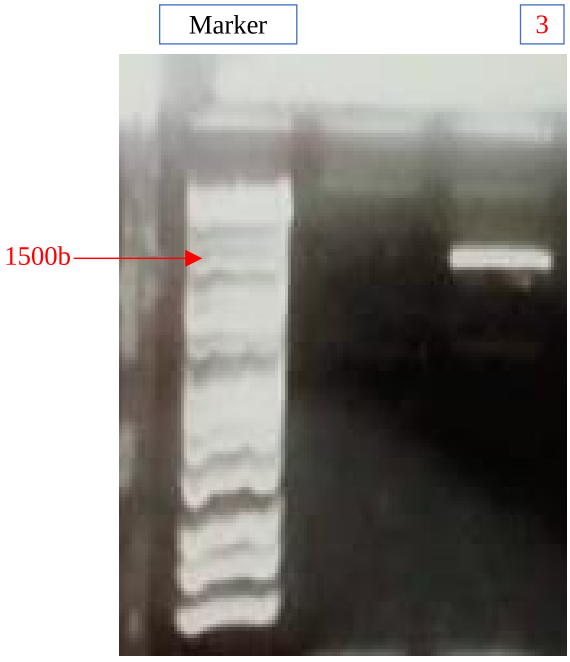

Figure 1. Electrophoresis Pattern of PCR Product of CYP2A6. Marker: DNA Molecular Marker 100 bp Size

Then product pcr is sequenced. Sequencing CYP2A6 is shown in the following figure.

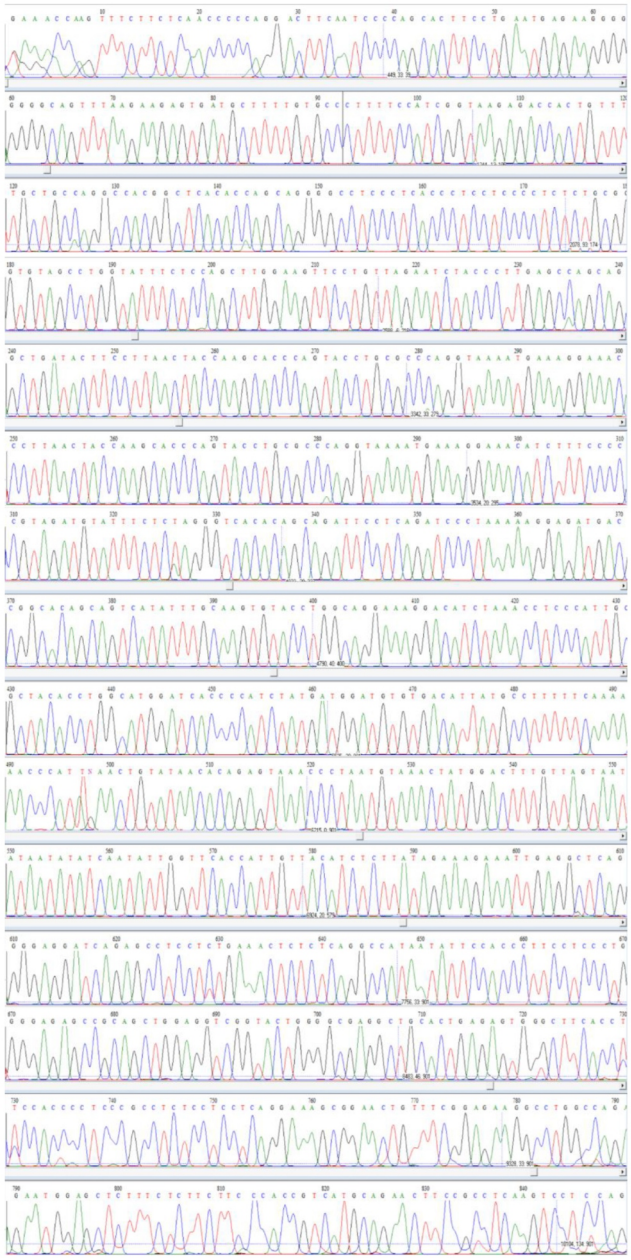

Figure 2. Sequencing Chromatograph Result for CYP2A6"

In this study, two out of ten samples were excluded because they did not give good results. The sequence of DNA showed nucleotide changes at several site for CYP2A6 compared to the NCBI database. The most common nucleotide changes are C to A. Table 2 shows the nucleotide positions and changes that occur in each sample.

Table 2. The Position of CYP2A6 Base Nucleotide Changes

Base Nucleotide Changes	*SAMPLE*								*Position in the CYP2A6*
	1	2	3	5	6	7	8	10	
C-A	-	-	V	V	V	V	-	V	7610
C-A	V	-	V	-	V	V	-	-	7629
G-C	-	-	-	-	V	-	-	V	7661
C-A	V	V	V	V	V	V	-	V	7715
C-A	V	V	V	V	V	-	-	-	7731
G-C	-	-	-	V	V	-	-	-	7739
T-A	-	-	V	V	-	V	-	-	7752
C-A	-	-	-	-	V	V	-	-	7788
C-A	V	V	V	V	V	V	-	V	7824
C-A	-	V	V	V	-	V	-	-	7869
C-T	-	-	-	-	-	V	-	V	8040
G-T	V	V	V	-	-	-	-	-	8375
C-T	-	V	V	-	-	-	-	-	8377
G-C	V	-	V	-	-	-	-	V	8379
A-C	V	V	V	-	-	-	V	-	8405

DISCUSSION

In our study, it was found that there were fifteen nucleotide sites or positions in CYP2A6 has changed, nucleotides C to A at position 7715 and 7824 were the most commonly found in the samples. The nucleotide changes in this study occurred in the intron area of the CYP2A6 gene. In general, changes in nitrogen bases may or may not have an effect on the protein product, depending on many factors. One of them is the location of the mutation will affect the protein product. The effect of mutations on non-coding areas in the DNA strand sequence, generally rarely has a direct phenotypic effect on the protein product. Mutations in introns that are non-coding areas will have an effect if their position in the regulatory area will affect the transcriptional level or translational level if the intron mutation occurs in the splicing region.[7]

Hekim et al. (2019) reported that there is some connection between mutation in the xenobiotic enzyme gene coding (CYP2D6 and CYP1A2) with the turkish male infertility and thus maybe CYP2A6 play a role in the male infertility.[8]

Limitation

The sample size is very insufficient but at least the variation that we found in this study can be used as base for the next study about this kind of subject with bigger sample size.

CONCLUSION

The conclusion of this study is that there are CYP2A6 variations that are found only in infertile male patients.

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