The frequency of Y chromosome microdeletions in infertile men of Vellore cohort

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The frequency of Y chromosome microdeletions in infertile men of Vellore cohort

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Abstract. Many male infertility cases are because of genetic and environmental factors and most of them are idiopathic. About 10-20% of azoospermic patients are showing the microdeletion in Y-chromosome. The azoospermia factor or AZF region at the Yq11 position which consists of genes those are necessary for spermatogenesis. In Y chromosome microdeletion, deletion in AZFa region is very rare. The aim of our study is to find out the frequency of microdeletions in Y chromosome particularly in AZFa region in azoospermic men of Vellore cohort. For this study, we collected 10 azoospermic patient and 10 control men samples from the Sandhya hospital, Vellore. In this study, we mainly focused on AZFa region to analyze the frequency of microdeletions in Y chromosome using SY82 (264bp) and SY83 (275bp) STS markers. There was an absence of microdeletion in patient samples for SY82 and SY83 markers of AZFa region. We concluded that sample size should be increased to confirm our results.

1. Introduction

Male infertility alludes to a male's inability to bring pregnancy in a prolific female [1]. Y chromosome microdeletion (YCM) is the second most successive hereditary reason for male infertility. It is a group of the hereditary issue brought on by missing genes on the Y chromosome [2]. Many men with Y chromosome microdeletion showed no side effects and had ordinary existences. There are mainly four regions present on Y chromosome i.e. AZFa, AZFb, AZFc, and AZFd responsible for Y chromosome microdeletion. AZFa microdeletion is found very rare [3]. Its size is 1MB and it has two candidate genes USP9Y and DBY. AZFb region is located in the middle region of Yq11 where a proximal piece of AZFb comprises of vast repeated sequences sorted out in palindromes, yet a large portion of it is the sequence of single copy [4]. At the point when both markers sY254 and sY255 are deleted, a finding of complete deletion of the AZFc region can be made. A few reviews have demonstrated that the AZFc deletion example is somewhat consistent,in spite of the fact that not generally indistinguishable. The most successive deletion sort is the AZFc region deletion trailed by AZFa, AZFb and AZFbc deletion [5]. Deletions which are distinguished as AZFabc are doubtlessly identified with irregular karyotype. Yq microdeletion testing is helpful for diagnosing infertility in men.

AZFa region deletion was observed very less frequently in the men who are infertile and were also associated with highly severe testicular phenotype with completely absent germ cells. It also contains three candidate genes which are USP9Y, DBY and UTY. They represent high homology with interval or deletions which may cause spermatogenic impairment. The main role of these genes in the human spermatogenesis is not known. It is also not clear about which of them may be responsible for the

phenotype of AZFa. Thecomplete AZFa deletion removes ~792 kb of the DNA sequences, including the two candidategenes [6]. AZFa genes deletion analysis in a vast number of men who are infertile is characterized by the highly defined spermatogenic alteration. Both the genes USP9Y and the DBY may also cause testiculopathies, but the gene DBY only appears to be the major candidate of AZFa.

YCM diagnosis grants the cause for the patient's azoospermia to be built up and to detail a prognosis [7]. Genetic counselling is required to provide information about the danger of conceiving a child with weakened spermatogenesis. Men with YCM will pass on their defect in their male offspring after going through ICSI treatment [8]. The testing of Yq microdeletion is done using PCR and involves the use of various STS markers for the detection of deletion occurred on Y chromosome. Therefore, in the present study we performed molecular diagnosis for AZFa region using STS-PCR.

2. **Materials and Methodology**

2.1 Study population

This study was done with the 10 azoospermic patients and 10 healthy controls between the age group of 18 to 45 years by performing semen analysis according to WHO, 2010 guidelines [9]. All of those individuals who were taken in the group of control were the healthy male without any symptoms of infertility or urological disease. They were recruited from the Sandhya hospital in Vellore, Tamil Nadu. The study was further approved by the ethical committee of Vellore Institute of Technology (VIT), Vellore. The sheet of the data collection from volunteers included the information of the population such as family history, age, BMI, smoking habits and physiological features. Informed consent form was given to volunteers. Genetic counselling gave some clue that mental state of the patient which indicated that up to some extent even depression, tension or the state of unhappiness may lead to the condition of infertility.

2.2 Genotype Determination

The extraction of the DNA was carried out from the 2ml intravenous blood sample which was collected in EDTA vacutainer. And it was further stored at -20°C for molecular analysis. This extraction was done by using standard lab protocol [10]. We have quantified DNA sample using Nano drop, 2000. We also qualified those DNA samples using Agarose gel electrophoresis technique. The STS primers SY82 and SY83 were used. The product size of SY82 primer was 264bp and for SY83 product size was 275bp. The sequence for SY82 forward primer was 5´-ATC CTG CCC TTC TGA ATC TC-3´and reverse was 5´-CAG TGT CCA CTG ATG GAT GA-3´. The sequence for the SY83 primer was 5´-CTT GAA TCA AAG AAG GCC CCT-3´ and reverse was 5´-CAA TTT GGT TTG GCT GAC AT-3´. The conditions used in PCR were such as- initial denaturation phase was at 95ºC for 5 minutes, denaturation phase was at 94ºC for 1 minute, the annealing temperature was 54ºC for both primers, the extension was done at 72ºC for 1 minute and final extension was at 72ºC for 10 minutes followed by thirty-five cycles. The PCR products were then analyzed for Y chromosome microdeletion by electrophoresis on 2% agarose gels. The absence of band in any STS markers indicated microdeletion by comparing it with the control. Sample showing deletions were tested again to confirm the results.

3. **Results and Discussion**

The patient's mean age was 33±6.7 (mean ± SD) years (range: 18-45 years) at the time of inclusion and all had primaryin fertility. There was no microdeletion found in SY82 and SY83 of AZFa region in azoospermic patients. Example of SY82 PCR products in AGE was shown in figure 1. In previous studies, it is reported that the deletion of the single the frequency of AZFa deletion is almost double (11 vs 5 %) in Indians than western population [11, 12]. Therefore, we assumed that more sample size should be needed to confirm our results.

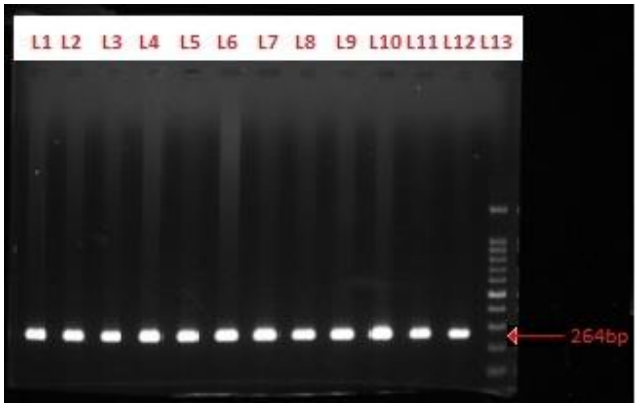

Figure 1. PCR products on a 2% of AGE for SY82 (Product size-264 bp)
(L1- L12: Patient's DNA samples, L13- 100 bp DNA ladder)

4. Conclusion

In a summary, this study showed the absence of Yq microdeletion in AZFa region in azoospermic men of Vellore population. Our findings are disagreeing with previous reports performed in different societies and ethnic groups in India. So, it is needed to screen more sample size with Yq microdeletions in azoospermic patients during diagnosis for male infertility and it should be carried out before ART. This will help to find out the etiology of male infertility.

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