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IMPACT OF CHROMOSOME ABERRATIONS AND MICRODELETION OF Y-CHROMOSOME INMALE INFERTILITY - A CROSS-SECTION COHORT STUDY IN NEPALESE POPULATION

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ABSTRACT

Socially, reproductive health is an important impact for human life. Male infertility includes genetic and epigenetics factor, WHO suggests that more that 9% human population all over the globe suffering from such disorder. There is scanty of information in the literature on male infertility in Nepalese population with reference to cytogenetic and molecular genetics. Hence, the present study has been designed with the aims to determine the frequency of chromosome aberrations as well as on molecular genetics analysis using STS markers for microdeletion of Y- chromosome with multiplex polymerase chain reaction in azoospermic and oligospermic infertile male (n=50) and compare with aged match controls. Cytogenetic findings reveals the significant variation in the frequency in both structural i.e. balanced translocation 46,XY,t (13;14) (q34;q22) in 2.0 % cases and numerical karyotype (47,XXY) in 10% cases with respect to controls. There is lack of mutation was observed in AZFc region of Y- chromosomes in male infertile cases, suggesting may beeither be due to less sample size or enhance DNA repair mechanism in next phase of the cell-cycle of meiotic II cell-division during spermatogenesis.

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INTRODUCTION

Infertility is commonly define as the difficulty to gets pregnancy even after one year of regular, unprotected intercourse. World Health Organization (WHO) suggests, that 9% of couples around the globe face challenges with fertility and male factors contribute to 50% of sub-fertility, while female factors co-contribute in 20% of cases (Katz, 2017 and Boivin *et al*., 2007). Approximately, 15% of couples globally suffer from infertility and the primary reasons for male include abnormal semen characteristics leads to clinical conditions like azoospermia, oligospermia, asthenozoospermia, teratozoospermia, and oligoasthenoteratozoospermia, occurring during spermatogenesis (Massart, 2012). Genetics factors includes variable frequency (5.8%) in structural and numerical chromosome aberrations in different population (Van Assche, 1996). The structural abnormalities of the Y-chromosome, such as deletion, ring chromosome, and isochromosome, may leads to vary phenotypic changes. For instance, the deletion of the sex-determining region on the short arm of the Y-chromosome affects testis differentiation, causing individuals to exhibit a female phenotype with streak gonads. Additionally, Y-microdeletions of azoospermic factor (AZF) regions a, b, and c on the short arm of the Y-chromosome shows variation in the frequency (7-8%) between two different population that lead to interference in male infertility (Saxena Ajit, 2019 and dos Santos, 2013).

European Academy of Andrology (EAA), suggests using six specific sequence-tagged sites (STS) markers have demonstrated a success rate for detection is 90% for Y- microdeletions in infertile males, while, 10-15% infertile males shows normal karyotypes (Sen, 2013 and Godoy, 2014). Therefore, the present study has been designed with the aims to carry out cytogenetic (chromosomal abnormalities) and molecular genetics study in synergistic manner to explore the etiopathology of male infertility in Nepalese population.

MATERIALS AND METHODS

To carry out analytical cross-sectional study, blood samples (1.0ml) were collected from clinically diagnosed cases of total of n=100 individuals of aged group between 24 to 50 years of male infertility (azoospermic and oligozoospermic) after written consent of the patient and equal number of normozoospermic act as controls having normal at least one birth were recruited for the present study. The study is approved by Institute Ethical Committee (IEC).

Cytogenetic study in Infertile Cases: Chromosomal analysis were carried out 0.5ml of peripheral blood in 5.0 ml of PB-MAX™ karyotyping media, supplemented with 300-µl of phytohemagglutininact as a mitogen followed by incubation at 37°C

in 5% CO2 (ESCO) for 72 hours. A 45ul of 0.08 µg/ml colcemid was then added to arrest cells at the metaphase after 68 hours of incubation. All chemicals including media were procured from Gibco (Thermo fisher) USA. GTG banded karyotypes were developed from well-spread metaphases (at least 50) from each cases, stained, and analyzed under oil immersion BX53 microscope, (Olympus, Japan), using GENASIS Software (Version 7.2, Applied Spectral Imaging, Israel) and chromosome abnormalities were analyzed according to ISCN 2016 guidelines.

Analysis of microdeletion of Y chromosome: After isolation of genomic DNA for this study was extracted using the total DNA extraction kit (Spin Star). Two multiplex assays were employed to assess Y - chromosome microdeletions within the AZF deletion regions. Primer mixes A and B were prepared, each containing specific primers for AZFa, AZFb, and AZFc regions reactions were performed using a specified thermal cycler program, including initial denaturation (heat activation) at 95^0C for 15 min, denaturation at 94^0C for 30-Sec, annealing at 57^0C for 90-Sec, elongation at 72^0C for 1 min, and final extension at 72^0C for 10 min. PCR amplified products were performed on a 2.0 % on agarose gel agarose gelelectrophoresis (in 1x TBE buffer) at 70V for 2 hours (in 1x TBE buffer) and bands were visualize after staining on gel Doc System (BioRad USA).

RESULTS

A total of 100 individuals that comprise 50 cases with male infertility (azoospermic and oligospermic primary infertile) and equal number of normozoospermic control subjects (males of proven fertility) were examined for cytogenetic and Y-chromosome microdeletion.

Table 1. Karyotype showing frequency (%) of structural and numerical chromosomal abnormalities in infertile men

Types of Variations	Karyotype	Cases [n (%)]
No chromosomal abnormalities	46,XY	44[50(88%)]
Numerical abnormalities	47, XXY	5[50(10%)]
Balanced carrier translocation	46, XY,t(13;14)(q34;q22)	1[50(2%)]

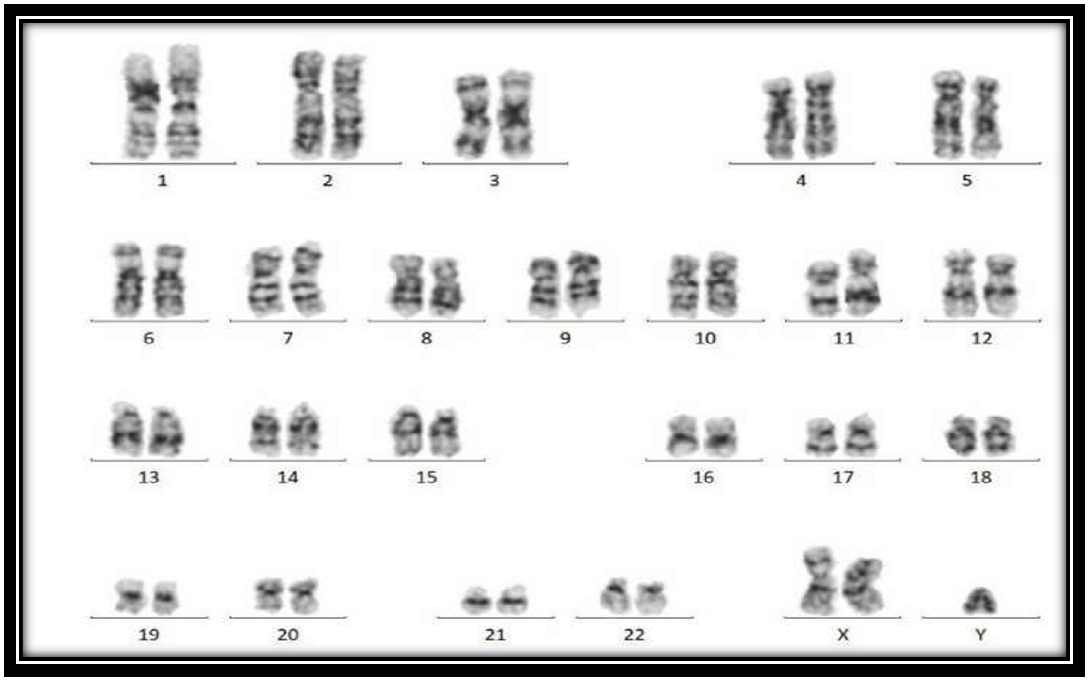

Figure 1. GTG banded karyotype showing 47, XXY (Klinefilter syndrome) in male infertility

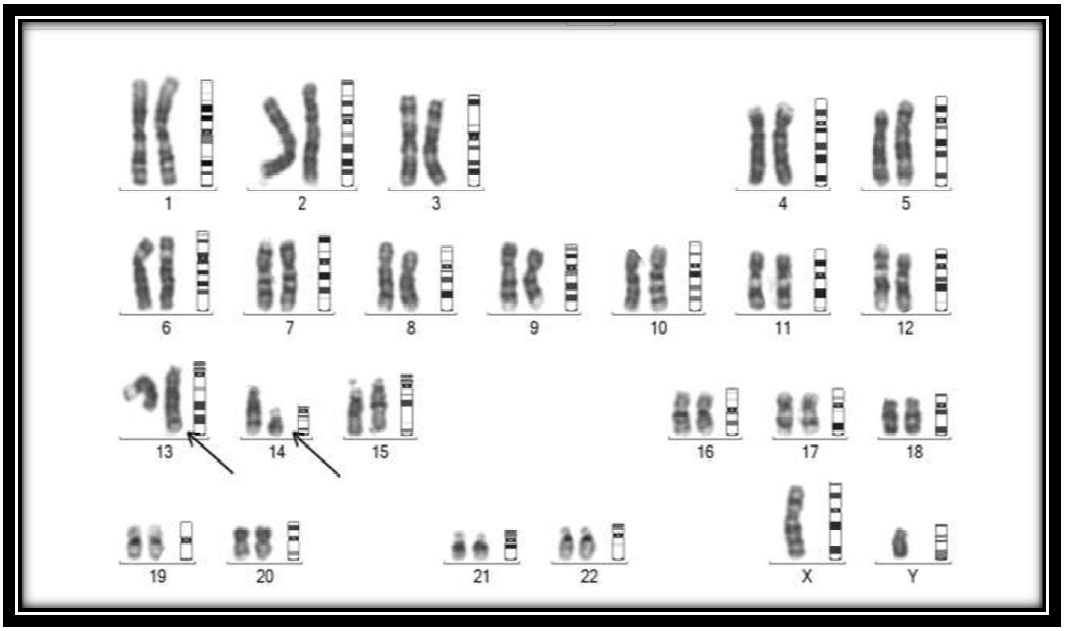

Figure 2. GTG banded karyotype showing balanced translocation 46, XY,t(13; 14) (q34; q22)

Among, 50 infertile male 18(36%) were azoospermic, 32(64%) were oligospermic. Of these 50 cases normal Karyotype of 46, XY was observed in 44 cases, chromosomal abnormality of 47,XXY-Klinefelter syndrome in 5 cases (Figure-I), balanced carrier translocation of 46, XY, t(13;14)(q34;q22) in one infertile man with severe oligospermia was observed. The present study based on multiplex PCR in Y- chromosome microdeletion in Nepalese population showing lack of deletion (mutation) in AZF region as shown in Figure-3.

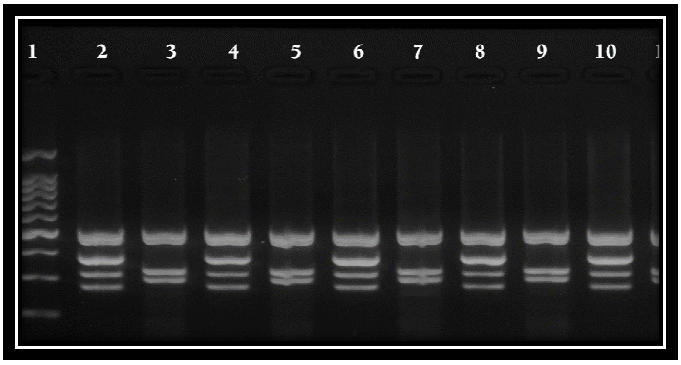

Figure 3. Multiplex polymerase chain reaction showing lack of changes (mutation) using specific STS markers.in microdeletion of Y- chromosome in male infertility (lane1-100bp ladder, lane 2-5 (azoospermic) and 6-10 (oligospermic) cases

DISCUSSION

The etiopathology of male infertility is highly complex due to involvement of genetic and epigenetic factors. Cytogenetic, in the present study showing both structural and numerical variation with different frequency in the infertile Nepalese male population. These genetic factors significantly contribute for abnormal semen parameters. It has been known over the last 20 years that the prevalence of chromosomal abnormalities is higher in infertile men and is inversely related to the sperm count (Czepulkowski, 1992). The results showed an inverse correlation between chromosomal anomalies and sperm count with spermatozoa bearing chromosome aberration may cause abnormal embryonic development leading to recurrent pregnancy loss or miscarriage in first trimester of pregnancy (Carlo,2001). Infertility, azoospermia, tall stature, and testicular atrophy are the phenotypic hallmarks of Klinefelter's syndrome, which has a 0.1% prevalence in the general population. Chromosomal analysis is crucial in determining the origin of this condition (Pandiyan, 1996). In this study, we observed five azoospermic men with Klinefelter syndrome - 47, XXY. Earlier study of Saxena (2019) also showed the significant correlation between high frequency mosaicism (46, XY/XYY) and microdeltion of AZF in Indian population (Saxena Ajit, 2019). The additional X chromosome of Klinefelter syndrome impacts on the mechanism of spermatogenesis, that affects testicular development, Leydig cell insufficiency and Sertoli cells apoptosis regulation (Jyothy *et al*., 2002). The main genetic cause of Klinefelter syndrome, is the presence of extra copy of X chromosomes due to non-disjunction event of sex-chromosomes, arises in the mother during oogenesis in meiosis I or II, whereas in the father (spermatogenesis), it can only arise only in meiosis I of the cell-division (Li *et al*., 2015). Mapping of chromosomes define specific functions of gene (s) involving specific break-points during spermatogenesis and reciprocal translocations are more likely to have abnormal pregnancies and present study may also evident that such chromosomal abnormalities act as carrier to interfere with ifertility (Bonomi, 2017). Although, various balanced translocations of different chromosomes such as t(1;19), t(3;13), t(1;9), t(9;10), t(9;3), t(1;4), t(7;8), t(3;6), t(1;11), t(1;10), t(3;18), t(7;9); t(7;14), t(7;17), (13;19), t(6;17) have been reported in male infertility (Zhang, 2015 and Sreenivasa *et al*., 2013). Based on current research, pre-gestational infertility is linked to 7q31 and 7q36 breakpoints, while gestational infertility is linked to 7q21.2, 7q22, and 7q32 break points (Suganya, 2015). Infertile men with carriers of del (13;14) frequency were approximately 10-fold higher than a newborn survey with missed abortions, stillbirths, and offspring with a congenital malformation (Sreenivasa *et al* 2013). Robertsonian translocations are inherited from paternal, nearly 40% shows *de novo* translocations due to rearrangements of genes in meiosis II (Suganya *et al* 2015). However, in present study, balanced translocation 46, XY, t(13;14)(q34;q22) may act as carrier has been reported reported first time in Nepalese population. According to the Practice Committee of the American Society for Reproductive Medicine (ASRM), the prevalence of YCMs (Y chromosome microdeletions) might be as high as 2 % in the general due to heterogeneous population of unselected men (Ching *et al*., 2012). According to a 2008 report, the frequency of AZF microdeletions among infertile men was less than 2.5% in Sweden, Germany, and Austria, whereas it was 10% (the highest) in Australia, China, and Brazil (Medicine, 2015). A comparative study carried out across Asia among patients with idiopathic azoospermia or severe oligozoospermia showed frequencies of 19.4% in China, 10.6%–11.7% in Taiwan, 15.8% in Japan, 9.6%–12.0% in India [5], 3.2% in Saudi Arabia, 3.3% in Turkey, and 2.6% in Kuwait. In a 2012 study of Y chromosome microdeletions in 115 patients in Iran, 1.7% showed deletions in the AZFc and AZFb regions. Akin *et al*. (2011) reported Y chromosome microdeletions in 7 patients (3.93%) among 178 infertile men. They were detected in the AZFc and AZFa regions (Krausz, 2013). A study conducted in Japan with 162 infertile males showed seven (4.3%) cases were positive for Y-microdeletion (Damdinsuren *et al*., 2022). Two other studies conducted in Egypt showed 4 out of 100 infertile men (4%), and 0/40 infertile males (0%) had absence of AZFc region on the Y chromosome (Wang *et al*., 2016). Along with EAA-recommended STS markers, each ethnicity population has to standardize with additional markers to increase the Y microdeletion detection rate. Present study based on multiplex PCR showing lack of microdeletions, specifically targeting the AZFa, AZFb, and AZFc regions due to selection of STS markers or less sample size during inclusion and exclusion criteria of the participants or may be unknown hidden environmental factor contributing in semen. The present study enhances our knowledge to understand the complexity between genetics and epigenetic factors influencing reproductive health.

CONCLUSION

The present study concludes that routine genetic screening of all infertile males to identify the minimize risk of transmission of chromosomal abnormalitiesand microdeletion of Y-chromosome to the offspring becomes essential. Interestingly, if the male partner has an abnormal autosomal karyotype then immediately genetic counseling should be required to the couple and recommended for assisted reproductive technique. To the best of our knowledge, present study of clinical genetic have great significance in the society and is reporting firsttime in Nepalese population.

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