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Study on Y- Chromosome Microdeletion of AZFc sY239, sY242 and sY254 Loci among Bangladeshi Infertile Male

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Abstract

Background: Infertility is a global health issue implicated to sociocultural, environmental, health care practice and genetic factors in both male and female individuals. Oligozoospermic and azoospermic conditions reported to be associated with Y chromosome microdeletions (YCDs) in various loci. **Objective:** The purpose of the present study was to investigate Y chromosome microdeletions in azoospermic individuals attending fertility clinics, focusing on the SRY239, SRY242, and SRY254 alleles. **Methodology:** This cross-sectional study was conducted in the Department of Physiology and Molecular Biology at Bangladesh University of Health Sciences, Dhaka, Bangladesh involving 20 infertile males. Appropriate procedures were followed to address ethical aspects. Demographic and clinical data were collected using structured questionnaire. One milliliter blood sample was collected for DNA extraction and downstream analysis. AZFc markers SY239, SY242 and SY254 alleles were determined by standard polymerase chain reaction (PCR). Data were expressed as number (percent) and figures as appropriate. A value < 0.05 was taken as level of significant. **Results:** Age (years) range of the 20 adult infertile azoospermic male was 25-50 with mean (±SD) 39 ±8. Of the 20 subjects 15 (75%) was smoker, 5 (25%) hypertensive and 2 (10%) diabetic. By profession their distribution includes day labors 4 (20%), businessman 8 (40%), garments workers 4 (20%), hawker 2 (10%) and teacher 2 (10%). Among 2 (10%) garments workers show their AZFc fragments deletions. Of the 20 men, azoospermia factor region (AZFc) SRY239 and SRY254 allele deletion were present in 4 individuals. The AZFc variant allele SRY242 was normal in all 20 men. Of the 4 individual present the SRY239 and SRY254 2 were obese. **Conclusion:** Y-chromosome microdeletion AZFc region allele markers SRY239 and SRY254 are present in azoospermic male but the AZFc SRY242 was of wild type. Presence of candidate allele detection did not show any relationship with study variables possibly due to small number of samples. To reach conclusive finding of AZFc mutations affecting male infertility, study need to be confirmed by recruiting substantial number of cases and carrying out the investigation at the early stage. [*Bangladesh Journal of Infectious Diseases, June 2024;11(1):22-29*]

Keywords: Male Infertility; Y-chromosome; Microdeletion; AZFc; Azoospermia

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Introduction

Infertility is a global health issue, affecting approximately 8.0% to 10.0% cases of couple's worldwide[1]. Magnitude of infertility found to vary widely in developed countries compared to those in developing and least develop countries. Cultural, socioeconomic and environmental factors reported to play major role in the prevalence of infertility[2]. Report from the World Fertility Survey, demonstrated almost similar rates of infertility in South Asia countries: Bangladesh 4%, Nepal 6%, Pakistan5% and Sri Lanka4% (Kumar et al., 2007)[1]. From 1990 to 2019, the global fertility rate reported to fall from 3.2 to 2.5 live births per woman. Presently close to half of the world's population lives in a country where lifetime fertility is below 2.1 live births per woman. The decline in fertility ranged from 1.9 births per woman in Central and Southern Asia to 0.1 births per woman in Australia and New Zealand, Europe and Northern America. In sub-Saharan Africa, the region with the highest fertility levels, the total fertility rate dropped from 6.3 to 4.6 births per woman. Fertility levels also found to fall in Oceania (from 4.5 to 3.4), Northern Africa and Western Asia (from 4.4 to 2.9), Latin America and the Caribbean (from 3.3 to 2.0), and Eastern and South-Eastern Asia (from 2.5 to 1.8)[3].

The causes of infertility reported to differ in respect to cultural, environmental and socioeconomic background[4]. The cause of infertility refers to (1) the female factor, (2) the male factor, (3) combined factors and (4) unexplained infertility. Female and male factors found to be responsible in 57% and 3% cases respectively. In 25% cases both male and female were responsible as combined factor infertility; remaining 15% contribute were unexplained infertility group[4]. Study of infertility by Malekshah[5] demonstrated that male factor responsible for 38.8% and female factors 34.7% cases. Among infertile couples about 14.6% cases both partners were implicated and 11.8% of cases no cause could be ascertained[5]. It was observed that as oligozoospermia, astheno- spermia, oligoastheno-spermia and terato-spermiatogether contributed in 40.3% cases of male infertility[6].

Age at menarche, marital age, age of first conception, and number of conceptions and live births are some of the biological factors assumed to determine fertility. The age group of 25-30 years was the most vulnerable as they represented 52% of primary and 51.42% of secondary infertility respectively[7]. In contrast to this, the educational and occupation status, household per capita annual income, family type, and use of birth control measures are the social factors that affect the fertility pattern of a population. Among the social factors that affect the fertility status of a population, preference for male child is one of the most important. The preference for a male child among the parents in the Indian society is one of the most important reasons leading to the explosion of population[8-10].

Race or ethnicity is a determinant of infertility and racial disparities account for a significant proportion of poor health outcomes overall[11]. The infertility among Black Haitian and Black African women found to be evenly distributed compared than to the White and Black American women. With regard to comparisons with racial/ethnic subgroups, Black American women had a higher frequency of infertility secondary to anovulation/PCOS (56.5%; P¼.001) compared with White American (39.8%), Black Haitian (25.5%), and Black African women (21.3%)[12].

While fertility is closely associated with effective cohabitation indicated by regular marriage, then delayed marriage is likely to strongly impinge on fertility[13]. Late marriage is one of the prevailing universal trends where people decide to get married at later ages. Cast system (67.1%), education (47.1%), idealism phobia (62.1%), and economic factor (77.1%) respectively found to responsible for late marriage[14]. By the early 1990s, median marriage age rose to its highest level in the 20th century, for both women and men. Studies revealed that age at marriage is an important social factor[15-17]. The fertility rate decreases when age at marriages increases as a result it shortens the reproductive span due to which complication arise in achieving pregnancy[18]. One Studied reported that about 65.7% respondents answered positively in accord that late marriages cause infertility[14].

Fine tuning of luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E2), testosterone, and sex hormone-binding hormone (SHBG), are demonstrated to play vital roles in the process of spermatogenesis and sperm maturation[19,20]. Several population-based studies[21-23] reported that circulating levels of sex hormones demonstrated association with sperm concentration, motility, or morphology[24-26]. The exact reason for this inconsistency remain unknown, but most of the studies were limited by small sample sizes and inadequate control for potential confounding [e.g., age, body mass index (BMI), smoking, and alcohol consumption]. Studies reported that abnormalities of LH and FSH levels were the commonest hormonal problems being present in 79.1% and

26.8% respectively among infertile males. Testosterone and prolactine level disorder was found in 5.1% and 12.1% respectively in male infertility[27]. The aim of this study was to investigate Y chromosome microdeletions as determined AZFc region SRY239, SRY242 and SRY254 alleles in a group of azoospermic subjects attending a fertility clinic.

Methodology

Study Settings and Study Population: This is a cross-sectional descriptive study was conducted in the dept. of Applied Laboratory Sciences. The study was conducted in the Department of Physiology and Molecular Biology at Bangladesh University of Health Sciences (BUHS), in collaboration with Harvest Infertility Care Limited, in Mirpur, Dhaka. The Study carried out from January 2019 to December 2021. After addressing the ethical issues, required number of samples was collected randomly from 20 Bangladeshi Infertile males associated with azoospermia, oligozoospermia in the age group 20-50 years from Harvest Infertility Care and Aalok Health Care Limited. Patients visited the fertility centers were approached following standard ethical practice. Inclusion Criteria: Infertile males with azoospermia, oligozoospermia. Exclusion Criteria: Males with normal reproductive characteristics. 20 samples were included in the study. Data was collected through Structured Questionnaire with informed consent. Questionnaire includes variables like BMI, co-morbidity factors (DM, HTN), smoking habit, professions, type of physical activity and duration of conjugal life, etc. Socio demographic data including age, BMI, body weight, height, and blood pressure, history of hypertension, diabetes, HTN and smoking was collected from their medical analysis report.

Blood collection and Laboratory analysis: For this study about 2ml of blood was taken into a tube containing EDTA (10ul) mixed thoroughly and preserved in at -20 degree Celsius for future DNA extraction and subsequent experimentation. Before extraction brings the sample to room temperature to avoid hemolysis as well as to ensure quality of DNA. After obtaining the informed consent 1ml of venous blood was collected in a micro- centrifuge tube containing 1 mg of EDTA. The whole blood was preserved at - 20DegreeCelsius until analysis. Genomic DNA was extracted by standard methods using QIAGEN DNA extraction KIT. The PCR program used was one cycle at 94°C for 3 minutes followed by 35 cycles of 94°C for 30 seconds, 59°C for 30 seconds and 68°C for 30 secondss. This was followed by one extension cycle at 68°C for 5 minutes. The PCR products were separated by electrophoresis on 2% agarose gel. A 100bp DNA ladder was loaded with PCR products to estimate band size. The gel was stained with ethidium bromide and photographed.

Selection of Y-Chromosome Deletion Sites: Deletions within the male-specific region of the Y-chromosome, known as Y- Chromosome Microdeletions (YCMs), are present in as many as 5% and 10% of severe oligospermic and azoospermic men, respectively. These microdeletions are distinguished by which segment of the Y chromosome is absent, identified as AZFa (the most proximal segment), AZFb (middle), and AZFc (distal). The reported prevalence of YCMs within the world's populations of infertile men displays vast heterogeneity, ranging from less than 2% to over 24% based on region and ethnicity. AZFc is the most commonly identified YCM, and its phenotypic presentation provides for the highest chance for fertility through artificial reproductive techniques. Conversely, deletions identified in the subregions of AZFa, AZFb, or any combination of regions containing these segments, are associated with low probabilities of achieving pregnancy[28].

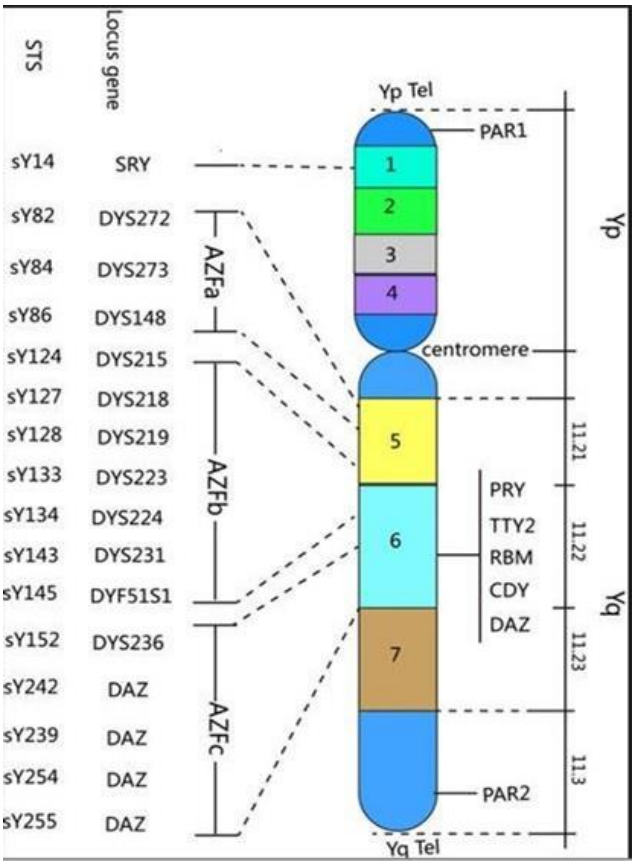

Figure I: Y-Chromosome Deletion Sites with specific STS[29]

Primer Selection for Y-Chromosome Microdeletion: DNA extraction from whole blood was performed by using chemicals and commercially available kit. Polymerase Chain Reaction (PCR) performed according to the standard protocol for analysis of microdeletion in AZFc region of the Y chromosome. Different types of STS markers are used to amplify AZFc region during PCR, for example; SRY239, SRY242 and SRY254 markers are used to identify the fragments AZFc deletion of[29]. This marker was confirmed by nucleic acid analysis according to standard protocol. In this study, 3 STS marker was used for the amplification of AZFc region. The following primer sets were used to amplify the target region in DNA.

Table 1: Primer Sets for Amplifications of Target Region

SL No.	AZFc Locus	Primer	Product Size (bp)
1	**sY239**	F:TACAGTCGGACGCGT CCCTCCATTCATCTTCCCTTTTGAAGG R:CTGGTCCGTACTACCGTGCGATGCAAGTCGCAGGAAATCT	241
2	**sY242**	F:TACAGTCGGACGCGTCCCTCACACAGTAGCAGCGGGAGTT R:CTGGTCCGTACTACCGTGCGTCTGCCACTAAACTGTAAGCTCC	273
3	**sY254**	F:TACAGTCGGACGCGT CCCTCGGGTGTTACCAGAAGGCAAA R:CTGGTCCGTACTACCGTGCGGAACCGTATCTACCAAAGCAGC	420

Results

Age, BMI, duration of marriage, area of residence, co-morbidity and personal habit were given below. Age range of the 20 azoospermic volunteers was 25-50 years. Mean(±SD) age (yrs) of them was 39 (±8) years. Of the 20 volunteers 14 (70%) come from urban and 6 (30%). Among them 8 (40%) were involved in business, day laborer 4 (20%), garments workers 4 (20%), teacher 2 (10%) as well as hawkers were 2 (10%). Duration (years) of marriage in one individual was 5yrs of the rest marital duration 8-13 years 6 (30%) had duration above 11 years. Of the 20 subjects 15 (75%) was smoker, 6 (30%) hypertensive and 3 (15%) diabetic subjects respectively; two volunteer had to this condition. According to Asia Pacific BMI report among 20 individuals 11 (55%) were obesity, 6 (30%) overweight and about 3 (15%) shown their normal BMI (Table 2).

Table 2: Characteristics of the Study Subjects (N=20)

Variables	Values
Mean Age±SD (yrs)	39.0±8.0
Body Mass Index (kg/m^2)	
• Normal Weight (3)	15.0%
• Over Weight (6)	30.0%
• Obese (11)	55.0%
Co-morbidity	
• Diabetes Mellitus (3)	15.0%
• Hypertension (6)	30.0%
Duration of conjugal life(yrs)	8 to 13
Type of Physical Activity	
• Low	25.0%
• Moderate	45.0%
• High	30.0%

Results were expressed as mean ± SD and percentages appropriate.

According to Asia Pacific Guideline of BMI we were expressed normal (BMI ≤ 18.5-22.9); over weight (BMI ≤ 23-24.9); obese (BMI ≥ 25) among the subjects. Physical activity: Low (sedentary work); Moderate (street vendor); High (industry ironman).

AZFc variant allele's analysis: Of the 20 men 4 (20%) demonstrated the presence of azoospermia factor region (AZFc) SRY239 and SRY254 allele deletion. The AZFc variant allele SRY242 was absent in all 20 men and only 5.0% shown azoospermia with infection according to their medical report. Among garments workers about 2 (10.0%) had their AZFc fragment deletions and their working environment was extremely hot condition coincidently. They were seeking for medical treatment for fertility issues for 6 years and no history of consanguinity was revealed.

Table 3: Results of AZFc Candidate Alleles Analyses

ZFc Candidate Allele	Present	Absent
sRY239	20%(4/20)	80% (16/20)
sRY242	0% (0/20)	100% (20/20)
sRY254	20% (4/20)	80% (16/20)

Result were expressed as number (percent)

AZFc SRY239 and SRY254 deletion were present in 4 (20%) of volunteers. AZFc SRY242 variant allele was absent in all 20 volunteers. Two (10% of the total) volunteers demonstrated present with AZFc. SRY239 and SRY254 variant allele used to work in readymade garments factory as iron man. Among the volunteers 15% were hypertensive, 20% were smoker and 50% were diabetic. As well as their BMI indicates among 4 volunteers 10% were overweight and 10% were obese respectively.

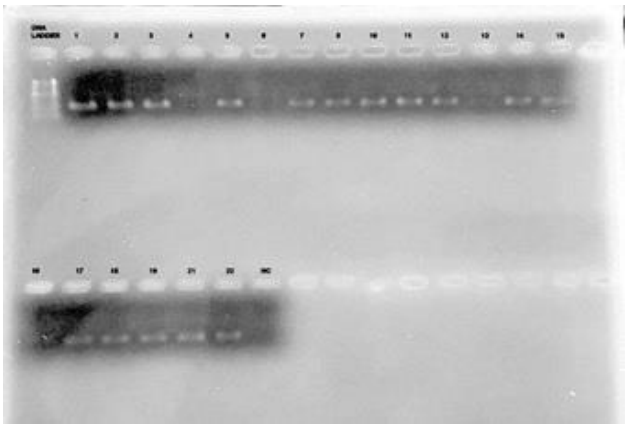

Figure II: Image of Y-Chromosome Microdeletion in Region C (STS 239 & 254)

Here, subjects 4, 6, 13 and 16 shows the complete deletion of Y-chromosome in region C (AZFc) with primer Sequence Tagged Sites (STS) 239 and 254.

Discussion

Male and female sterility is not uncommonly seen all over the world. The scenario in Bangladesh is of no differences which however are almost similar in numeric number like other Asian countries. Data are lacking regarding the cases of recording sterility in both male and female in Bangladesh. In our study the frequency of Y-chromosome microdeletion in region C is 20% and this data compare with other studies (Table 4).

The present study was aimed to look into the AZFc region common gene variations implicated in male infertility. The volunteers seeking fertility treatment for 7-8 years. Study variables retrieved from medical records and mainly Analyzed AZFc common variant allele; which demonstrated 2 out 20 of them presence of STS239 and STS254 mutation. But STS242 was absent. Although in other studies (Table 2) detection of three candidate alleles detection was demonstrated, but in the presence study STS242 allele was of wild type. Which is clearly showed a little genetic variation. However, this need to be confirmed involving large study samples and looking into its clinical associations. The present study not only limited to demonstration of one of the three AZFc fragments, but also lack of data regarding the previous STD exposure and immunogenic data, e.g; antibody against previous susceptible viral infection. The two volunteers demonstrated to have STS239 and STS254 of AZFc fragments were azoospermic. There were also found to be obese 20% in this study. This present study appeared to be involving small number of patients and little data derived.

Table 4: Percentage of Yq- AZFc Microdeletion in Different Study

Study Title	Year	Percentage of Yq AZFc Microdeletion	Population
Study on Y-Chromosome Microdeletion of AZFc sY239, sY242 and sY254 locai in Bangladeshi Male Infertility	2021	20% (4/20)	Bangladeshi
Prevalence of Y chromosome microdeletion in azoospermia factor subregions among infertile men from West Bengal, India	2021	9.32% (11/118)	West Bengal, India[39]
Molecular Analysis for Azoospermia Factor Microdeletions in the Y chromosome for Azoospermic and Severe Oligospermic Infertile Iraqi Patients.	2020	37.5% (24)	Iranian[40]
Ferrtilization and embryonic development of azoospermia with AZFcmicrodeletion	2015	6.2% (21/337)	Japanese[41]

Study Title	Year	Percentage of Yq AZFc Microdeletion	Population
A novel universal multiplex PCR improves detection of AZFc Y- chromosome microdeletions	2014	7.6% (41/540)	Chinese[42]
Y chromosome microdeletions in infertile men: prevalence, phenotypes and screening markers for the Indian population	2013	Deletions of AZFc were at highest frequency (46.6 %) 1636 total sample	Indian[43]
The frequency of Yq microdeletion in azoospermic and oligospermic Iranian infertile men Detection of sperm in men with Y chromosome microdeletions of the AZFa, AZFb and AZFc regions	2013	80% (50)	Iranian[44]
Detection of sperm in men with Y chromosome microdeletions of the AZFa, AZFb and AZFc regions	2003	53.8% (42/78)	American[45]

Future studies are warranted to look into genetic involvement along with exploration of other risks factors, attributed to male fertility in Bangladeshi population, with previous studies. We have compared these findings with other articles. The DNA from STD pathogens was detected in semen of 45 of 241 asymptomatic men seeking an infertility investigation [18.7%; cytomegalovirus (CMV) 8.7%, human papillomavirus (HPM): 4.5%, Herpes simplex virus (HSV) 4.5%, Human herpesvirus type 6 (HHV6) 3.7%, Epstein–Barr virus 0.4%, hepatitis B virus 0% and C. trachomatis 2.5%][30].

Past history of Mumps and Rubella was reported in 6.0% of male infertility. A little recent report demonstrated 21.4% in fertile men had history of STD exposure[31]. Obesity has been linked to sperm count and quality leading to affecting fertility: higher BMI associated with decrease in sperm count and motility[32]. Anuploidy, Down and Klinefelter syndrome cases were known to be infertile. However, there are sporadic reports which demonstrated DS causes fertility problem also[33].

After Klinefelter's syndrome, Yq-microdeletions are the second most frequent and important spermatogenesis genetic disorder in male infertility. Previous studies have reported that Yq microdeletions changed from 1.0% to 55.0% between infertile men, but most studies have revealed this ratio below 15%[34]. The molecular investigation of AZF loci allows the recognition of many genes that have a major role in spermatogenesis. The candidate genes within the AZFc region include Four DAZ copies (Deleted in azoospermia), three BPY2 copies (Basic Protein on Y chromosome 2). Also, two copies of Y chromo domain, Y-linked CDY (CDY1a and CDY1b)[35]. DAZ is encoding an RNA- binding protein essential for spermatogenesis[36]. AZFc deletions may be less pathogenic, so the chance of TESE in the case of complete AZFc deletion is 50.0% cases[37]. Controversy in Y chromosomal microdeletions of infertile men arises from this fact that Yq microdeletions will be transmitted by ICSI and cause the infertility problem in sons[38].

There are some limitations of this study which are small number of samples, only AZFc region allele was determined. Oligozoospermic male were not included. Volunteers were not tested at the presentation

Conclusion

Y-chromosome microdeletion allele markers SRY239 and SRY254 are present in infertile men in small frequency. This is significant genetic check for male infertility problems in Bangladesh. To reach conclusive comment of AZFc mutation affecting infertility study need to be expanded by recruiting substantial number of cases and carrying out the genetic study at the early stage. Proper identification of AZFc microdeletion is equally important in case of successful assisted reproductive techniques like, ICSI and IVF. Azoospermia is closely associated with chromosomal abnormalities. The level of testosterone, human luteinizing hormone, and FSH in men with azoospermia showing abnormal karyotypes provides a clinical reference for genetic counseling and assisted reproduction. Study involving statically adequate numbers of individuals and testing associated markers alleles suggest in other studies is warranted. Microdeletion markers alleles should be tested at the early stage to conclusively comment on its effect on sperm count of the individuals.

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Conflict of Interest
No competing interests exist by the authors.

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Contribution to authors:
Hazera Akter Mukta, Md. Ashiqur Rahman: Conception and design, or design of the research. Hazera Akter Mukta, Md. Ashiqur Rahman: The acquisition, analysis, or interpretation of data; conceptualized and designed the overall study. Hazera Akter Mukta, Md. Biplob Hossain, Fatima Anjum Faruquee, Shohanur Rahaman: Involved in data collection; Sadia Islam, Md. Ashiqur Rahman: Drafting the manuscript or revising it critically for important intellectual content. Md. Biplob Hossain, Shohanur Rahaman, Fatima Anjum Faruquee: Involved in data input and data cleaning. Hazera Akter Mukta, Sadia Islam, Md. Ashiqur Rahman: Conducted data analysis. Md. Ashiqur Rahman, Hazera Akter Mukta: Drafted the manuscript. All authors reviewed and approved the final manuscript

Data Availability
Any questions regarding the availability of the study's supportin g data should be addressed to the corresponding author, who can provide it upon justifiable request.

Ethics Approval and Consent to Participate
This study was approved by the Institutional Review Board of Bangladesh University of Health Sciences (BUHS). All participants were informed about the study and they gave their written consent before inclusion in the study.

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