Y- CHROMOSOMAL MICRODELETIONS IN A LOCAL INFERTILE MALE POPULATION OF PAKISTAN

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Background: The aim of this study was to determine occurrence, frequency and pattern of microdeletions of the long arm of Y chromosome within the AZFa, AZFb, and AZFc subregions in patients with idiopathic azoospermia, and correlate the microdeletions with clinical phenotypes to determine the most important subregion for screening in a sample of local population of Pakistan. **Methods:** The patients were selected from the Male Infertility Clinic, Services Hospital, Lahore. They included 53 cases, 25–45 years of age with male factor infertility and 53 age matched controls with normal semen parameters. Blood and semen samples were obtained from all the participants. We performed semen analysis, polymerase chain amplification of 8 DNA loci on the long arm of the Y chromosome using 8 pairs of primers according to guidelines by the European Academy of Andrology. Plasma FSH and testosterone levels were also determined to assess the endocrine status**. Results:** No Y-chromosomal microdeletion could be detected in the infertile or fertile males included in the study. One of the two samples of EAA Quality Control Schemes showed deletion in AZFc region and all the three (AZFa, AZFb, AZFc) were missing in the second sample. Mean serum concentrations of FSH and testosterone in both groups of subjects were found to be within the normal range. However, FSH levels were significantly higher in infertile males than the fertile males whereas testosterone levels were significantly lower in infertile men as compared to fertile subjects. **Conclusion:** These results suggest a much lower Yq deletion frequency than previously thought, even among strictly selected patients with idiopathic azoospermia or oligozoospermia in a sample of local population of Pakistan.

Keywords: Infertile males, Y-Chromosome, Microdeletion

INTRODUCTION

Fertility is defined as the capacity to conceive and produce offspring. Infertility is the state of a diminished capacity to conceive and bear offspring. The current clinical definition of infertility is the inability to conceive after 12 months of frequent coitus.[1,2]

Male infertility is a major health problem today and according to certain estimates, among the infertile couples visiting infertility clinics, 40–50% of males in the reproductive age group have qualitative or quantitative abnormalities in sperm production.[3–6]

Male infertility is associated with a reduction in the quantity, motility or abnormal morphology of sperm. In about 50–60% of male infertility cases the aetiology can be identified but when the cause is unknown, it is referred to as idiopathic infertility.[6]

Normal sperm production, maturation and function are long and complex processes that require an appropriate hormonal environment but also well-balanced autocrine, paracrine, and juxtacrine signalling events between the various components of the male reproductive system.

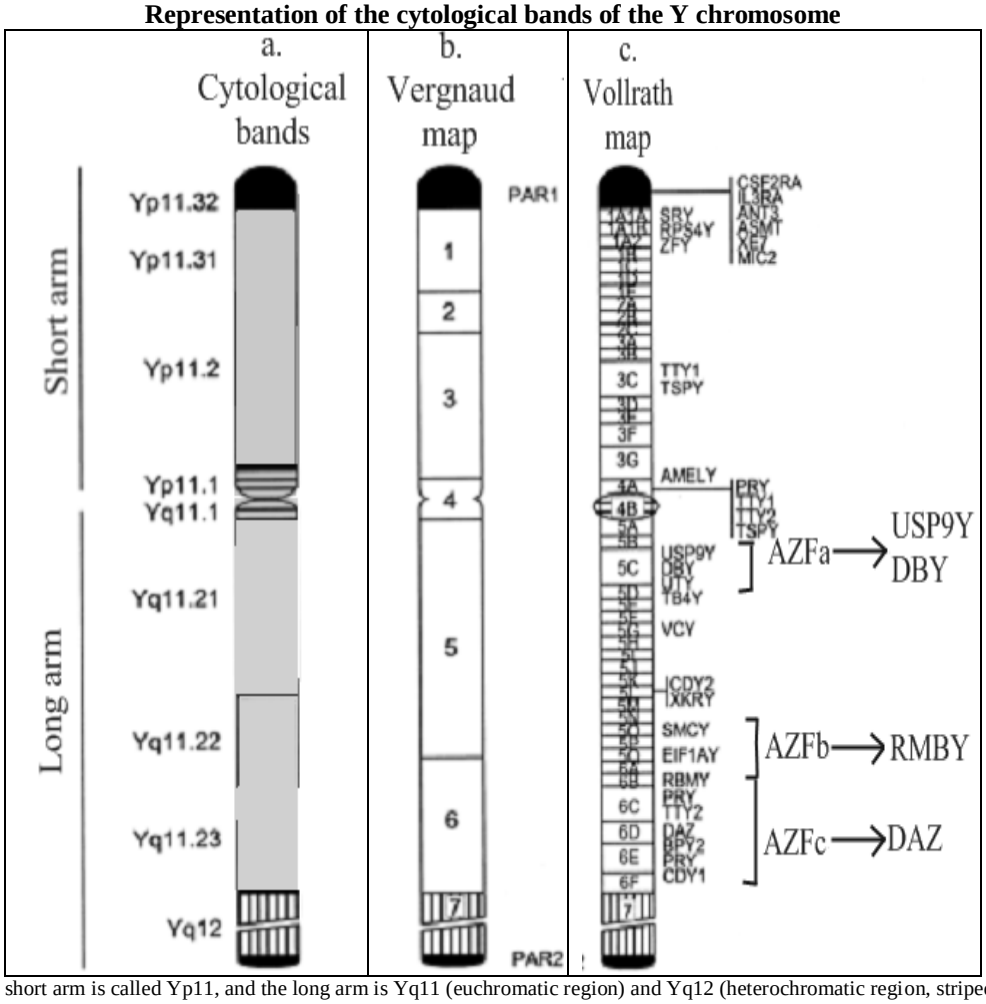
The mechanisms regulating the spermatogenic process are complex involving both genetic and endocrine control factors. The endocrine control factors involve the hypothalamic- pituitary-gonadal axis while the genetic control has been mapped to the gene and gene families located on the long arm of Y chromosome.[6,7]

In a number of cases of male infertility no obvious causative etiological factor can be identified (also known as idiopathic or unexplained infertility).

The Y chromosome, the one that carries the gene that makes a man male, is unlike any other. It's the only chromosome in the human genome that is present only in males. The Y chromosome is the smallest of all in the human genome with 60 million base pairs. The genetic information on the Y chromosome is important for male sex determination and for normal spermatogenesis.[8,9]

The Y chromosome consists of a short [Yp] and a long [Yq] arm (Figure-1). The ends of the long and short arms are known as pseudoautosomal regions [PARs] which pair with the X chromosome during meiosis. The intervening large segment of the Y chromosome known as the male specific Y [MSY] contains many genes that are involved in spermatogenesis. This is also the non-recombining region of the Y chromosome [NRY].[3]

Representation of the cytological bands of the Y chromosome



a: The short arm is called Yp11, and the long arm is Yq11 (euchromatic region) and Yq12 (heterochromatic region, striped).
b: The seven intervals of the Vergnaud map of the Y chromosome, where intervals 1–4 span the short arm and the centromere, intervals 5 and 6 span the euchromatic region, and interval 7 spans the heterochromatic region.
c: The 43 interval map of the Y chromosome. On the *right* are represented a list of genes mapped to the Y chromosome, the localization of AZF regions and the corresponding candidate genes [Modified from Foresta *et al*[3]].

The genes critical for spermatogenesis are located on the long arm of the Y chromosome in deletion interval 5 and 6. This region is referred to as the azoospermia factor (AZF), as the most severe phenotype associated with this is azoospermia.[10] The AZF region was originally subdivided into three-nonoverlapping loci-AZFa, AZFb and AZFc, deletions of which are associated with spermatogenic failure.[6,10]

AZFa region contains ubiquitin- specific protease 9 [USP9Y] and dead box on the Y [DBY].

AZFb region involves RNA binding motif on the Y [RBMY].

AZFc region has the 'deleted in azoospermia [DAZ]' gene cluster as an important candidate gene.[3,6]

Yq Microdeletions

DNA deletions have been shown to be common mutational events and the size of these deletions may vary from a single to several megabases of DNA. Deletions small enough to be detected only by techniques such as STS–PCR or Southern hybridization are known as microdeletions.[8,9]

These microdeletions occurring in Y chromosome might be playing an etiological role in unexplained male factor infertility.

MATERIAL AND METHODS

Our study included 53 infertile males between the ages of 25–45 years. Patients visiting local hospitals and infertility clinics were included in the study. Samples from 53 age matched males with normal semen parameters (as documented by semen analysis) were used as controls. Strict inclusion and exclusion criteria were observed while selecting the two study groups.

Semen Analysis

Both macroscopic and microscopic examination was done. On naked eye inspection, appearance, colour,

volume, viscosity and liquefaction time was observed. On microscopic examination, sperm concentration, motility and morphology were observed. Maklers chamber was used to determine the sperm count. The diagnosis of azoospermia/oligozoospermia was made on the basis of semen analysis according to guidelines established by WHO.[11]

Microdeletion Analysis

Screening of Y chromosome deletions were carried out according to the guidelines and protocol jointly recommended by the European Academy of Andrology and the European Molecular Genetics Quality Network.[12] Whole blood was used for DNA extraction. Polymerase chain amplification of 8 DNA loci on the long arm of the Y chromosome using 8 pairs of primers was carried out.

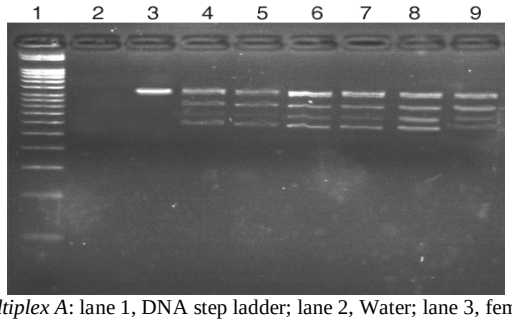
Hormone Assays

Follicle stimulating hormone (FSH), and testosterone concentrations were determined in the serum using standard immunoassays to evaluate the endocrine status of individuals involved in the study group.

RESULTS

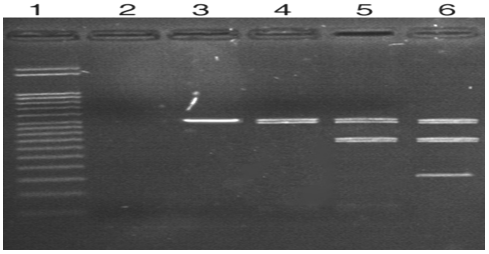
No Y-chromosomal microdeletion could be detected in these 106 males including both cases and controls. Testosterone levels were found to be significantly lower in cases as compared to controls while FSH levels were comparatively higher in cases.

PCR Analysis Results

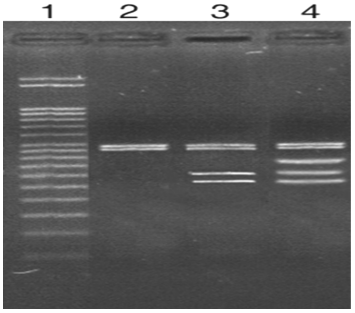


Multiplex A: lane 1, DNA step ladder; lane 2, Water; lane 3, female DNA; lane 4, DNA of normal fertile male; lane (5, 6, 7, 8, 9), infertile male patients.

Quality Control Analysis

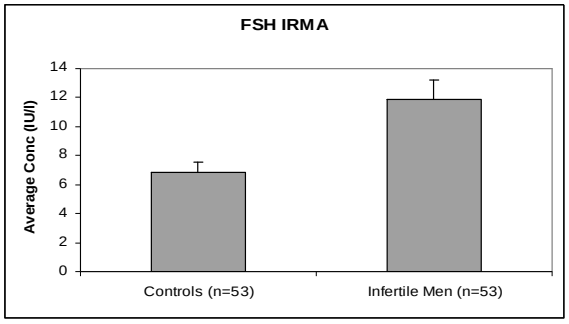


Multiplex B:
lane 1, DNA step ladder; lane 2, Water; lane 3, female DNA; lane 4, DNA of AZF a, b, c deleted patient; lane 5, DNA of AZF c deleted patient; lane 6, DNA of normal fertile male



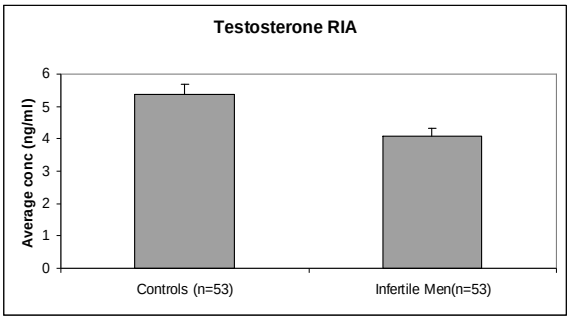
Multiplex A:
lane 1, DNA step ladder; lane 2, DNA of AZF a, b, c, deleted patient; lane 3, DNA of AZF c deleted patient; lane 4, DNA of normal fertile male.

Hormone Analysis



$p<0.05$

Serum FSH concentrations in Controls and Infertile Men



$p<0.05$

Serum Testosterone concentrations in Controls and Infertile Men

Table-1: Status of sperm count in infertile subjects

Status	Number	Percentage
Azoospermic	39	73%
Oligozoospermic (Sperm Count < 1 million/ml)	3	5.6%
Oligozoospermic (Sperm Count < 10 million/ml)	11	21.4%
Total	53	100%

Table-2: Y-Chromosomal microdeletion in infertile and fertile subjects

Group	Y-Chromosomal Microdeletion
Infertile Subjects (n=53)	Nil
Control Subjects (n=53)	Nil

Table-3: Serum testosterone and FSH concentration in infertile and control subjects

Hormone	Infertile Subjects (n=53)	Control Subjects (n=53)	*p*
Serum FSH (Mean±SEM) (IU/L)	11.90±1.32	6.86±0.64	<0.05*
Serum Testosterone (Mean±SEM) (ηg/ml)	4.08±0.25	5.38±0.3	<0.05*

* Statistically significant

DISCUSSION

The results of our study are in accordance with the data published worldwide. The average Yq microdeletion frequency of more than 3000 infertile males published is about 7%[13] however, the prevalence differs in a wide range from 1%[14] to 55%[15]. Wide variations in deletion frequency reported in previous published works could be caused by ethnic differences, different patient selection criteria and partly by methodological aspects.[16]

A study conducted in Germany reported similar results as no deletions were found even among strictly selected 97 infertile males[17] while in The Netherlands and Belgium deletions were found in 2.3% of 1627 infertile men[18] whereas another study confirmed Y chromosomal microdeletions in only 1% of subjects[19]. Another recent study conducted in Germany has concluded that frequency of AZF deletions is much lower in German population as compared to populations of other regions.[20]

Furthermore, the significantly raised FSH and lowered testosterone levels found in infertile subjects shown in the present study can be based on testicular hypofunction. In addition to altered spermatogenesis, raised FSH levels may be due decreased release of inhibin from the malfunctioning Sertoli cells while decreased testosterone levels based on defective role of Leydig cells.[1,2]

Infertile men suffering from idiopathic spermatogenic failure are at risk for Y chromosomal microdeletions. It is generally recommended that these men with severe male factor infertility should be screened for Y chromosomal microdeletions as a part of their pretreatment investigations as this is important from both medical and ethical point of view.[20,21]

There is some evidence that these deletions are passed from fathers to their sons by the use of assisted reproductive techniques such as IVF/ ICSI and thus the problem of infertility could be passed on to the next generation. Hence, genetic analysis is strongly recommended for infertile couples with male factor infertility opting for assisted reproduction.[21–24]

CONCLUSION

These results suggest a much lower Yq deletion frequency than previously thought, even among strictly selected patients with idiopathic azoo- or oligozoospermia in a sample local population of Pakistan. However, the study needs to be extended with respect to screening of more infertile patients and also exploring recently discovered subregions of Y chromosome that might be involved in spermatogenesis. Also the significant hormonal variation needs to be explored further.

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