Open Access
Full Length Article

ORIGINAL ARTICLE

Y Chromosome Microdeletions in Pakistani Infertile Men

Atif Mahmood[1], Syeda Nuzhat Nawab[2], Saima Ejaz[3], Masood Anwar Qureshi[4], Fauzia Imtiaz[5], Abid Azhar[6]

1. Associate Professor, Department of Physiology, Bhitai Dental and Medical College
2. Scientific Officer, Khan's Institute of Biotechnology and Genetic Engineering (KIBGE), University of Karachi.
3. Lecturer, Northern Border University, Arar, Saudia Arabia
4. Chairman/Head Physiology Department, Dow University of Health Sciences; Director, Institute of Basic Medical Sciences (DUHS)
5. Associate Professor, Department of Biochemistry, Dow University of Health Sciences
6. Director General, Khan's Institute of Biotechnology and Genetic Engineering (KIBGE), University of Karachi.

ABSTRACT

Objective: To determine the prevalence of Y chromosome microdeletions in Pakistani idiopathic infertile men, using multiplex polymerase chain reaction.

Patients and Methods: A case control study was conducted on the infertile male patients attending OPD's of Aziz Medical Hospital and National Center for Fertility Control, Jinnah Post Graduate Medical Center (JPMC), Karachi. A total of 220 primary infertile men, of which 150 (68.2%) had azoospermia, 40 (18.2%) had severe oligozoospermia and 30 (13.6%) had oligozoospermia and 220 fertile men as control group were studied. Six sequence-tagged sites: sY84 and sY86 for AZFa, sY127 and sY134 for AZFb, and sY254 and sY255 for AZFc were used for amplification of the azoospermia factor region of Y chromosome according to the recommendations of European Academy of Andrology and the European Quality Monitoring Network Group.

Results: Yq microdeletions were found in (12) 5.45% cases, while none in the control group (p =<0.007). All the microdeletions were found in azoospermics 12/150 (8.0%). Among patients with microdeletions, the AZFa region was found to be deleted in 1 (8.33%), AZFb in 2 (16.67%), AZFc in 6 (50%), AZFb+c in 2 (16.67%) and complete AZF deletions in 1 (8.33%) patients. Identification of Y chromosome microdeletions in Pakistani infertile males was found to have significant diagnostic and prognostic value and provides useful information for genetic counseling in patients choosing assisted reproductive treatments.

Key Words: Azoospermia, Azoospermia Factor, Male Infertility, Oligozoospermia, Polymerase Chain Reaction, Y chromosome microdeletions.

Author`s Contribution
[1]Conceived the topic of research and designed the study
[2]Literature review and manuscript writing
[3]Data Analysis and Discussion

Address of Correspondence
Dr. Atif Mahmood
atif_mahmood20@yahoo.com

Article info.
Received: Oct 16, 2016
Accepted: Feb 7, 2017

Cite this article: *Mahmood A, Nuzhat S, Ejaz NS, Qureshi MA, Imtiaz F, Azhar A. Y Chromosome Microdeletions in Pakistani Infertile. JIMDC. 2017; 6(1):14-21.*

Funding Source: *Nil*
Conflict of Interest: *Nil*

Introduction

Approximately 10–15% of couples are affected by infertility which come into consideration when the couples fail to conceive after unprotected coitus for more than one year. A male factor can be diagnosed in approximately 50% of them. The main causes of this disorder for men are associated with various factors, among them are genetic, physio-pathologic and anatomo-pathologic abnormalities, intense and prolonged physical exercises, aging, drugs, and even excessive time of sexual abstinence.[1] In almost 20% of cases in which male infertility cannot be attributed to any other cause, the role of genetic alterations is being increasingly scrutinized.[2] This form of infertility can be classified as a genetic disorder, where structural chromosomal alterations,

acquired or congenital, have been one of the main etiologic factors.[3] Little is known about the reproductive genetic disorders that cause disruption of spermatogenesis. Early cytogenetic studies showed that microscopic deletions in the long arm of Y chromosome are responsible for azoospermia and structural alterations in the Y chromosome have been the principal reason for male infertility.[4]

Numerous investigations have documented interstitial microdeletions in AZFc region in both azoospermics and mild oligospermics. Such microdeletions have been reported with varied prevalence in different populations. For example, the studies in Brazilian population reported both low (6.7%) as well as high (44%) prevalence of Yq microdeletions in the AZFc region in infertile males.[5,6] Likewise, low frequency (5.1%) as well high frequency (17.4%) has been shown in Chinese population.[7,8] Further the range of frequency of AZFc region deletion in Indian population has been 5.3% -15%.[9,10] AZFc deletions and partial AZFb deletions are associated with sperm retrieval in 50% of cases, while in cases of AZFb deletions, probability of finding sperm is virtually nil. However, retrieved sperms are found to be fully fertile in both Intra-cytoplasmic Sperm Injection (ICSI) and In vitro fertilization (IVF) procedures. ICSI allows partners of infertile men to become pregnant; however, it is possible that Y deletions may be transmitted to the male offspring. Substantial prevalence of Y deletions in infertile men and the potential risk of transmitting this genetic disorder to their offspring provide a compelling rationale for screening of infertile men prior to ICSI.[11]

The status of AZFc in the genetic involvement of male infertility has been documented in many countries with varying frequency. So far only two studies have reported Y chromosome microdeletions in Pakistani infertile men. One of these studies was conducted in Lahore and has not reported any microdeletions, whereas another study which was conducted in Islamabad reported an overall prevalence of 11.6% Yq microdeletions in Pakistani infertile men.[12,13] Thus, it becomes imperative to investigate the deletions of AZFc region localization in Pakistani population. The aim of this study was to detect genetic defect in male infertile patients attending infertility clinics and to evaluate it for etiological diagnosis in cases of idiopathic male infertility.

Patients and Methods

A case control study was conducted on the infertile male patients attending OPD's of Aziz Medical Hospital and National Center for Fertility Control, Jinnah Post Graduate Medical Center (JPMC), Karachi. The bench work was carried out at Khan Institute of Biotechnology and Genetic Engineering (KIBGE), University of Karachi from June 2012 to May 2013. A purposive non probability sample of 220 idiopathic infertile men with a normal karyotype aged between 21 and 60 years (mean ± SD = 33.54 ± 6.57) were screened for the presence of Y chromosome microdeletions. Two hundred and twenty age-matched (mean ± SD = 37.24 ± 5.65 years) fertile men with proven fertility and sperm count > 20 million/ml were considered as control group; also a female sample was used as negative control. Informed written consent was obtained from each and every subject included in the study and confidentiality and anonymity of the data was ensured. The study was approved by the Institutional Review Board (IRB) of Dow University of Health Sciences (DUHS).

A detailed history and physical examination was done on all the subjects. Semen analysis was performed according to normal standard parameters using the World Health Organization (WHO) criteria. Infertile men (n=220) were divided into 3 groups on the basis of sperm count: azoospermic (No spermatozoa), severe oligozoospermic (less than 5 million/ ml) and oligozoospermic (less than 20 million/ml). Five microlitre of venous blood was also collected in a tube containing Ethylene Diamine Tetraacetate (EDTA) as an anticoagulant for DNA extraction. Genomic DNA was extracted utilizing Phenol chloroform method. Polymerase Chain Reaction (PCR) was performed according to the standard protocol for analysis of the AZF region of the Y-chromosome. Three sub-regions were analyzed: AZFa, AZFb and AZFc, where sequence tagged site (STS) markers were used. As positive control, fertile men with naturally conceived children were used. These STS markers were suggested by the European Academy of Andrology which are able to detect 90% of the deletions in the loci of AZF.[2]

The PCR amplification reaction mix comprised a total volume of 15µL, which contained 1.5µg of 50ng/µL human genomic DNA as template, 1 µL of 2mM dNTP's (Fermentas), 5µL of 100 µM each of the forward and

reverse primers (Pennicon), 0.3µL of 50mM $MgCl_2$ (Geneaid) and 0.1µL of 5 units/µL Taq DNA polymerase (Geneaid), 1.5µL of 10X PCR Buffer (Geneaid) and 0.6µL of deiodinized water. The conditions for thermocycling were standardized for the sub-regions, utilizing a Thermo Hybaid *MBS 0.2S* PCR Thermal Cycler.

Two sets of Multiplex PCR were used; Multiplex A (sY86, sY127, sY254) and Multiplex B (sY84, sY134, sY255). In addition, two sets of primers (ZFX/ZFY and sY14) were used to amplify SRY and ZFY regions as internal controls as shown in Table 1. The cycling conditions for multiplex A PCR amplifications were: 35 cycles at 95ºC 78 ºC and 52ºC for 45 seconds each. Initial denaturation was done at 94 ºC for 4 min and final extension at 72 ºC for 10 min. The cycling conditions for multiplex B PCR amplifications were: 35 cycles at 95ºC, 78 ºC and 52ºC for 45 seconds each in cycle. Initial denaturation was done at 94ºC for 4 min and final extension at 72ºC for 10 min in both the multiplex. The amplified PCR product (15uL) was loaded in 2% agarose gel in 1x Tris-Borate EDTA (TE) Buffer and was run by gel electrophoresis in gel assembly for 30 minutes at 200 volts. The gel was stained with ethidium bromide (5ug/100ml) and the results were visualized by gel documentation system under UV light and photographed. The size of the DNA bands was identified by comparing with the molecular weight marker (100bp DNA ladder) (Fermentas, 0.5µg/µL) loaded in a separate lane. This analysis was performed at least three times on samples with microdeletions. In each multiplex PCR essay, one sample from healthy female was used as negative control, and healthy fertile male was used as positive control. Statistical analysis was carried out by the Statistical Package for Social Science version 16.0. The student's *t* test and Chi-square were used to determine the associations among the different parameters. P value of less than 0.05 was considered to be significant.

Results

Among total of 220 subjects of the study group, mean age of the patients was found to be 33.54 ±6.57 years whereas it was 37.24 years ± 5.65SD for the control group. The mean age of their wives was 28.15 years ± 4.63SD, whereas mean age in control group was 27.99 years ± 5.05 SD. The average time since the marriage in

Table 1. Sequence-tagged Sites (STS) used in the detection of Y chromosomal microdeletions

Multiplex	STS	Locus	Region	Sequence 5' to 3'	Bp
A and B	**ZFX/ZFY**	ZFX/ZFY	Xq34	F5' - ACC RCT GTA CTG ACT GTG ATT ACA C – 3'	**495**
			Yp22.3	R5' - GCA CYT CTT TGG TAT CYG AGA AAG T – 3'	
A and B	**sY14**	SRY	Yp11.3	F5' - GAA TAT TCC CGC TCT CCG GA – 3'	**472**
				R5' - GCT GGT GCT CCA TTC TTG AG – 3'	
A	**sY254**	DAZ	AZFc	F 5'-GGG TGT TAC CAG AAG GCA AA-3'	**380**
				R 5'-GAA CCG TAT CTA CCA AAG CAG C-3'	
A	**sY86**	DYS148	AZFa	F5' - GTG ACA CAC AGA CTA TGC TTC – 3'	**318**
				R5' - ACA CAC AGA GGG ACA ACC CT – 3'	
A	**sY127**	DYS218	AZFb	F 5'-GGC TCA CAA ACG AAA AGA AA-3'	**274**
				R 5'-CTG CAG GCA GTA ATA AGG GA-3'	
B	**sY84**	DYS273	AZFa	F 5'-AGA AGG GTC CTG AAA GCA GGT-3'	**326**
				R 5'-GCC TAC TAC CTG GAG GCT TC-3'	
B	**sY134**	DYS224	AZFb	F5' - GTC TGC CTC ACC ATA AAA CG – 3'	**301**
				R5' - ACC ACT GCC AAA ACT TTC AA – 3'	
B	**sY255**	DAZ	AZFc	F5' - GTT ACA GGA TTC GGC GTG AT – 3'	**123**
				R5' - CTC GTC ATG TGC AGC CAC- 3'	

study group was 6.98 years ± 4.85 SD and was 5.39 years ± 5.26 SD in control group (Table 2). The semen of the enrolled patients was analyzed for the number of sperms as per WHO criteria as shown in Figure 1.

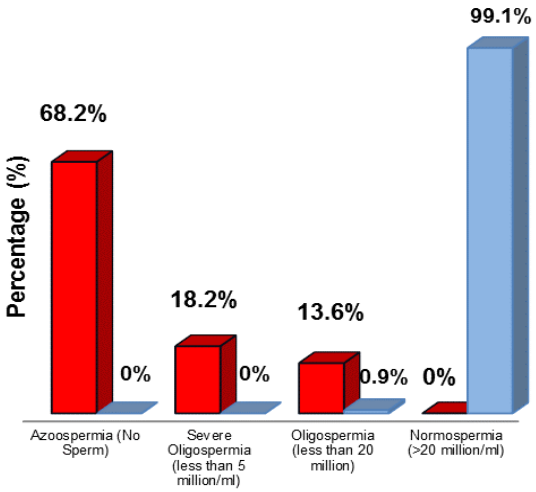

Figure 1. Distribution of the infertile men and healthy fertile controls on the basis of Sperm Counts (million/ml)

Amongst the total of 220 subjects (shown in Red) that were included in the study, 68.2% males (n=150) were found to be azoospermic i.e. having no sperm in their semen. About 18.2% cases (n=40) were found to be severely oligospermic i.e. having sperm count less than 5 million/ml, while 13.6% patients (n=30) were found to be oligospermic having sperm count less than 20 million/ml.

Amongst the control group (shown in blue), 99.1% (n=218) individuals had their sperm count greater than 20 million/ml while only 0.9% (n=2) were found oligospermic. The sperm counts for the two groups had significant difference with p value of <0.01. Table 3 shows that the microdeletions in the Y-chromosome were found in 5.45% cases (12 individuals out of 220) while no microdeletion was detected in controls. (p =<0.007). Amongst the microdeletions that were reported during the study, 2.73% were isolated AZFc deletions; 0.91% were AZF b and AZFb+c deletions each while 0.45% deletions were found in AZFa and AZFabc regions. These microdeletions were observed in azoospermics only. Of all the microdeletions, the AZFa region was found to be deleted in 8.33% (1/12), AZFb in 16.67% (2/12), AZFc in 50% (6/12), AZFb+c in 16.67% (2/12) and AZFa+b+c in 8.33% (1/12) patients. Figure 2 represents optimization of the primers of Multiplex PCR: 100 bp, Ladder; 1, Primer 1 (495 bp); 2, Primer 2 (475 bp); 3, Primer 3 (320 bp); 4, Primer 4 (274 bp); 5, Primer 5 (380 bp); 6, Primer 6 (326 bp); 7, Primer 7 (301 bp); 8, Primer 8 (123 bp). Figure 3 shows the absence of band of 380 bp in lane 5, 6 and 7 which corresponds to AZFc region. Band of 274 bp and 380 bp are absent in lane 8 and 9 showing deletion in the AZFb + c region whereas lane 10 shows complete deletion of the entire AZF region. Figure 4 shows the absence of band of 123 bp in lane 5, 6 and 7 which corresponds to AZFc region. Band of 301 bp and 12 bp are absent in lane 8 and 9 showing deletion in the AZFb + c region whereas lane 10 shows complete deletion of the entire AZF region

Table 2: Age of the couples and duration of marriage

Parameter	Cases (n=220)		Control (n=220)	
	Mean ± SD	95% CI (Lower – Upper)	Mean ± SD	95% CI (Lower – Upper)
Age of the patients (years)	33.54 ± 6.57	32.67 – 34.41	37.24 ± 5.65	36.49 – 37.99
Age of the wife (years)	28.15 ± 4.63	27.53 – 28.76	27.99 ± 5.05	27.32 – 28.66
Years since married	6.98 ± 4.85	6.34 – 7.63	5.39 ± 5.26	4.69 – 6.09

Table 3. Deletion rate and Distribution of observed microdeletions of AZF region on Y chromosome in infertile men

AZF region	Azoospermia (n=150)	Severe Oligozoospermia (n=40)	Oligozoospermia (n=30)	Total (n=220)	Deletion rate (%)
AZFa	1	-	-	1	0.45
AZFb	2	-	-	2	0.91
AZFc	6	-	-	6	2.73
AZF b+c	2	-	-	2	0.91
AZF a+b+c	1	-	-	1	0.45
Total (%)	12 (8%)	-	-	12	5.45

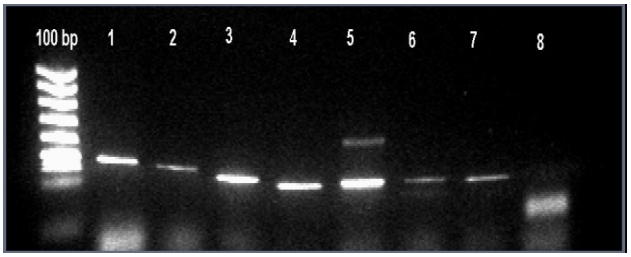

Figure 2: Optimized Multiplex PCR with all the eight primers

1	2	3	4	5	6	7	8	9	10

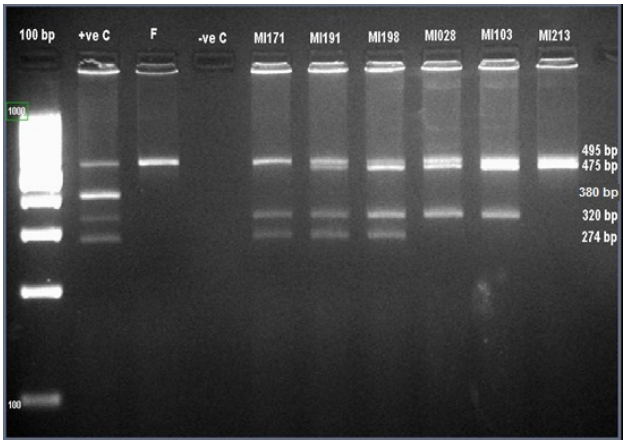

Figure 3: Multiplex A: lane 1, 100bp DNA Ladder; lane 2, DNA of normal fertile male as +ve Control; lane 3, female DNA; lane 4, water as –ve Control; lane (5, 6, 7), DNA of AZFc deleted patient; lane (8, 9), DNA of AZFb+c deleted patient; lane 10, DNA of AZFa, b, c deleted patient.

1	2	3	4	5	6	7	8	9	10

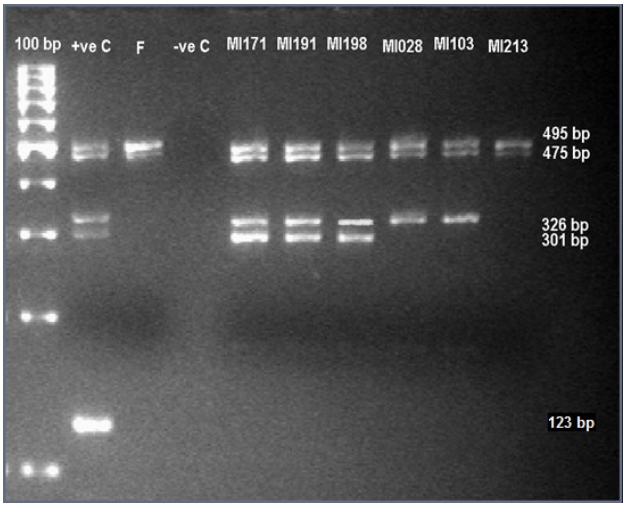

Figure 4: Multiplex B: lane 1, 100bp DNA Ladder; lane 2, DNA of normal fertile male as +ve Control; lane 3, female DNA; lane 4, water as –ve Control; lane (5, 6, 7), DNA of AZFc deleted patient; lane (8, 9), DNA of AZFb+c deleted patient; lane 10, DNA of AZFa, b, c deleted patient.

Discussion

Among numerous etiologic factors, genetics play a key role in male infertility with abnormal semen parameters.[14-16] Spermatogenesis is regulated by a number of genes on the Y chromosome and by autosomes that act at different stages of germ cell development.[16] Previous reports have revealed that Y chromosome microdeletions vary from 1 to 55% among infertile men all over the world, but most studies have reported an incidence below 15%.[2] In the present study, the estimated frequency was 8% among azoospermic patients and an overall prevalence of 5.45% which is well within the range of the published data. No microdeletion was reported in a study conducted in Lahore city in 53 idiopathic azoospermic infertile men while using European Academy of Andrology (EAA) recommended STS markers.[12] Another study conducted in Islamabad city for Y chromosomal microdeletions in oligozoospermic and azoospermic men reported an overall prevalence of 11.8% in 51 patients.[13] These deletions were found solely in non-obstructive azoospermic patients which is also the case in our study as no microdeletion was found in the oligozoospermic or severe oligozoospermic patients.

Wide variations in deletion frequency that are reported in previous published works could be caused by geographic and ethnic differences, genetic background, and environmental influences, different patient selection criteria, study designs, sample size and partly by methodological aspects.[17,18] However, there are studies that suggest that the major factor influencing deletion frequency was the composition of study population and ethnic or geographical differences apparently had no influence on it.[19,20] They found that the highest deletion frequency was in the group defined as idiopathic azoospermic with the incidence of 17 percent and the incidence decreases progressively with the inclusion of less severe phenotypes.[20] It seems that the discrepancy in reported deletion frequencies in Pakistani population could be explained by (i) differences in the sample size, and (ii) ethnic variations since our samples were collected from Karachi which is a metropolitan city and accommodates people from many different ethnic groups and races.

It has been reported that AZFc microdeletion constitutes a progressive and deteriorating effect on spermatogenesis leading to a steady and methodical decline in spermatogenesis and deterioration in sperm quality.[21] But, a clinical study of azoospermic and oligospermic men, did not agree to this concept as they found that the AZFc deleted men had their baseline sperm production potential stable over time.[22] It has been shown that chromosome Y microdeletions are much less frequent when sperm concentration is less than 2 million/ml.[23,24] This may be one of the reasons for the failure to detect Y microdeletions in the non-azoospermic groups which is also observed in our study where complete AZF deletion and the deletions of AZFa, AZFb, AZFb+c and all the AZFc deletions were all found only in azoospermic male. Deletions in AZFc region are the most commonly reported deletions among AZF microdeletions and its complete deletion is one of the most frequent genetics causes of severe male infertility. In a study conducted on 247 Saudi men with idiopathic azoospermia or oligospermia, 3.2% had Y chromosome microdeletion, consisted of 6 in the AZFc, 1 in the AZFb, and 1 in both AZFa and AZFc.[15]

AZFa contains single copies of DFFRY (USP9Y) and DBY (DDX3Y) genes. It is suggested that complete deletion of AZFa region may result in complete SCO syndrome and azoospermia.[2,4,25,26] Identification of deletions in this region is very important since it is impossible to retrieve testicular sperm for ICSI.[2,25] In our study, AZFa region was involved in a total of 8.33% (1/12) of the observed Y chromosome microdeletions using sY84 and sY86 sequence tagged sites. Deletion in this region was observed in combination with complete deletions of AZF region in one patient (8.33%). This is slightly higher than the percentage of deletions reported in literature in AZFa region, which is 2 -5%.[19,27] This higher percentage was also reported by the another study conducted in our country, in which one patient out of six with microdeletions showed AZFa deletion (16.67%).[13] A study conducted in India reported 2% AZFa region deletion.[28]

AZFb deletion was involved in 41.7 % of the total deletions of which AZFb alone was involved in 16.67 %. Selected STS markers for detecting deletions in AZFb region in the present study were located in the median and distal part of AZFb and in the most cases the deletion of sY127 and sY134 markers, indicated a complete deletion of the AZFb region which may cause SCO syndrome or spermatogenetic arrest resulting in azoospermia.[2] Two patients showed complete deletion of AZFb whereas two patients showed partial deletion in AZFb region. *RBMY* genes, including *RBM1* and *RBM2*, located in AZFb region, are specifically expressed in testis and germ cells. The gene encodes a RNA binding protein that localizes to the nucleus of all spermatogenic cell types.[25,29] Since several copies of these genes are located in the AZFb region, it is not clear whether the loss of the *RBMY* genes in men may result in male infertility or not, therefore, the role of deletion in these genes is not clear in the process of spermatogenesis.[30] Several reports have shown that complete deletions in AZFb region will have the same results as deletions in AZFa region for testicular sperm extraction (TESE).[2,25] Patients with complete or partial AZFb deletion in our study also showed maturation arrest in their testis biopsy; therefore, attempts for retrieving sperm were not successful.

Conclusion

Y chromosome microdeletions proved to be the second most common etiology among genetic causes of male infertility after Klinefelter's syndrome. However, men with microdeletions will presumably transmit the deletion, as well as the related fertility problem, to their sons. It is, therefore, very important not only to routinely screen for Yq microdeletion but also to offer proper genetic counseling to the couples particularly those who wish to undergo assisted reproductive techniques.

References

1. Pasqualotto FF. Investigation and assisted reproduction in the treatment of male infertility. Revista Brasileira de Ginecologia e Obstetrícia. 2007;29(2):103-12.
2. Simoni M, Bakker E, Krausz C. EAA/EMQN best practice guidelines for molecular diagnosis of y-chromosomal microdeletions. State of the art 2004. International journal of andrology. 2004;27(4):240-9.
3. Stankiewicz P, Lupski JR. Genome architecture, rearrangements and genomic disorders. TRENDS in Genetics. 2002;18(2):74-82.
4. Vogt PH, Edelmann A, Kirsch S, Henegariu O, Hirschmann P, Kiesewetter F, et al. Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. Human molecular genetics. 1996;5(7):933-43.
5. SaoPedro SL, Fraietta R, Spaine D, Porto CS, Srougi M, Cedenho AP, et al. Prevalence of Y chromosome deletions

in a Brazilian population of nonobstructive azoospermic and severely oligozoospermic men. Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas / Sociedade Brasileira de Biofisica,et al. 2003;36(6):787-93.
6. Arruda JT, Bordin BM, Santos PR, Mesquita WE, Silva RC, Maia MC, et al. Y chromosome microdeletions in Brazilian fertility clinic patients. Genet Mol Res. 2007;6(2):461-9.
7. Yang Y, Zhang SZ, Peng LM, Ding XP, Lin L, Wang J. Studies on molecular epidemiology of Y chromosome azoospermia factor microdeletions in Chinese patients with idiopathic azoospermia or severe oligozoospermia. Zhonghua yi xue yi chuan xue za zhi = Zhonghua yixue yichuanxue zazhi = Chinese journal of medical genetics. 2003;20(5):385-9.
8. Liu XZ, Tang YG, Zheng LX, Zhou BY, Liu H, Li MZ, et al. Chromosome abnormalities and Y chromosome microdeletions in patients with the azoospermia and cryptozoospermia. Zhonghua yi xue yi chuan xue za zhi = Zhonghua yixue yichuanxue zazhi = Chinese journal of medical genetics. 2010 Aug;27(4):460-2.
9. Mitra A, Dada R, Kumar R, Gupta NP, Kucheria K, Gupta SK. Screening for Y-chromosome microdeletions in infertile Indian males: utility of simplified multiplex PCR. The Indian journal of medical research. 2008 Feb;127(2):124-32.
10. Babu SR, Swarna M, Padmavathi P, Reddy PP. PCR analysis of Yq microdeletions in infertile males, a study from South India. Asian journal of andrology. 2002 ;4(4):265-8.
11. Van Landuyt L, Lissens W, Stouffs K, Tournaye H, Liebaers I, Van Steirteghem A. Validation of a simple Yq deletion screening programme in an ICSI candidate population. Molecular human reproduction. 2000;6(4):291-7.
12. Siddique A, Qureshi HJ, Siddique S, Husnain A. Y-Chromosomal microdeletions in a local infertile male population of Pakistan. Pak J Physiol. 2009;5(1):29-33.
13. Siddiqi S, Siddiq A, Majeed K, Mansoor A, Qamer R, Mazhar K, et al. Y-Chromosomal Deletions- a Risk Factor for Male Infertility. International Journal of Agriculture and Biology [Short Communication]. 2009;11(1):110-2.
14. Samli H, Solak M, Imirzalioglu N, Samli M. Genetic anomalies detected in patients with non-obstructive Azoospermia and Oligozoospermia. Med J Kocatepe. 2005;6:7-11.
15. Hellani A, Al-Hassan S, Iqbal MA, Coskun S. Y chromosome microdeletions in infertile men with idiopathic oligo- or azoospermia. Journal of experimental & clinical assisted reproduction. 2006;3:1.
16. Dada R, Gupta NP, Kucheria K. Cytogenetic and molecular analysis of male infertility: Y chromosome deletion during nonobstructive azoospermia and severe oligozoospermia. Cell biochemistry and biophysics. 2006;44(1):171-7.
17. Zhu YJ, Liu SY, Wang H, Wei P, Ding XP. The prevalence of azoospermia factor microdeletion on the Y chromosome of Chinese infertile men detected by multi-analyte suspension array technology. Asian journal of andrology. 2008 ;10(6):873-81.
18. Vutyavanich T, Piromlertamorn W, Sirirungsi W, Sirisukkasem S. Frequency of Y chromosome microdeletions and chromosomal abnormalities in infertile Thai men with oligozoospermia and azoospermia. Asian journal of andrology. 2007 ;9(1):68-75.
19. Krausz C, Forti G, McElreavey K. The Y chromosome and male fertility and infertility. International journal of andrology. 2003;26(2):70-5.
20. Krausz C, Rajpert-De Meyts E, Frydelund-Larsen L, Quintana-Murci L, McElreavey K, Skakkebaek NE. Double-blind Y chromosome microdeletion analysis in men with known sperm parameters and reproductive hormone profiles: microdeletions are specific for spermatogenic failure. The Journal of clinical endocrinology and metabolism. 2001;86(6):2638-42.
21. Simoni M, Gromoll J, Dworniczak B, Rolf C, Abshagen K, Kamischke A, et al. Screening for deletions of the Y chromosome involving the DAZ (Deleted in AZoospermia) gene in azoospermia and severe oligozoospermia. Fertility and sterility. 1997;67(3):542-7.
22. Oates RD, Silber S, Brown LG, Page DC. Clinical characterization of 42 oligospermic or azoospermic men with microdeletion of the AZFc region of the Y chromosome, and of 18 children conceived via ICSI. Human reproduction. 2002;17(11):2813-24.
23. Ferlin A, Moro E, Garolla A, Foresta C. Human male infertility and Y chromosome deletions: role of the AZF-candidate genes DAZ, RBM and DFFRY. Human reproduction (Oxford, England). 1999;14(7):1710-6.
24. McLachlan RI, O'Bryan MK. Clinical Review: State of the art for genetic testing of infertile men. The Journal of clinical endocrinology and metabolism. 2010 ;95(3):1013-24.
25. Sadeghi-Nejad H, Farrokhi F. Genetics of azoospermia: current knowledge, clinical implications, and future directions. Part II: Y chromosome microdeletions. Urology journal. 2007;4(4):192-206.
26. Krausz C, Quintana-Murci L, McElreavey K. Prognostic value of Y deletion analysis. Human Reproduction. 2000;15(7):1431-34.
27. Krausz C, Degl'Innocenti S, Nuti F, Morelli A, Felici F, Sansone M, et al. Natural transmission of USP9Y gene mutations: a new perspective on the role of AZFa genes in male fertility. Human molecular genetics. 2006;15(18):2673-81.
28. Mahanta R, Gogoi A, Roy S, Bhattacharyya IK, Sharma P. Prevalence of Azoospermia Factor (AZF) Deletions in Idiopathic Infertile Males in North-East India. Int J Hum Genet. 2011;11(2):99-104.
29. Elliot DJ, Millar MR, Oghene K, Ross A, Kiesewetter F, Pryor J, et al. Expression of RBM in the nuclei of human germ cells is dependent on a critical region of the Y

chromosome long arm. Proceedings of the National Academy of Sciences of the United States of America. 1997;94:3848-53.
30. Delbridge ML, Harry JL, Toder R, O'Neill RJW, Ma K, Chandley AC, et al. A human candidate spermatogenesis gene, RBM1, is conserved and amplified on the marsupial Y chromosome. Nature genetics. 1997;15:131-6.