VOLUME-6 | ISSUE-4 | APRIL - 2017 • ISSN No 2277 - 8179 | IF : 4.176 | IC Value : 78.46

ANALYSIS FOR THE DETECTION OF FREQUENCY OF Y CHROMOSOME MICRODELETIONS IN THE AZFa, AZFb and AZFc REGION IN DIFFERENT INFERTILE GROUPS OF NORTH EAST INDIAN POPULATION.

Biological Science

KEYWORDS: Male Infertility, Y chromosome microdeletions, AZF, Azoospermia, Cryptozoospermia, Oligozoospermia, Asthenozoospermia, Teratozoospermia Oligoasthenozoospermia, Asthenoteratozoospermia.

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ABSTRACT

The present study was undertaken to determine the frequency of AZF deletions in infertile men with azoospermia, cryptozoospermia, oligozoospermia, asthenozoospermia, teratozoospermia, oligoasthenozoospermia and asthenoteratozoospermia of North East India. In our study, Five hundred infertile patients and One hundred fertile men were screened for Y chromosome microdeletions. No microdeletions were detected in fertile men. Among 500 infertile men,123(24.6%) had azoospermia, 10 (2%) had cryptozoospermia, 103 (20.6%) had oligozoospermia, 112 (22.4%) had asthenozoospermia, 32 (6.4%) were teratozoospermia, 84 (16.8%) had oligoasthenozoospermia and 36 (7.2%) had asthenoteratozoospermia.According to our findings it was observed that the frequency in AZFc region is highest, followed by AZFa region and AZFc region in both blood and semen samples.

1. INTRODUCTION:

Infertility is a major public health problem with significant social, psychological and economic impact that affects 10 to 22% of couples in the human population. In about 50% of these couples, the problem lies in the male either solely or in combination with female factor (Foresta *et.al.,* 2001, Chandley *et.al.,* 1994). Male infertility can be considered to be a multifactorial syndrome that has a collection of different conditions with a variety of etiologies (Khabour *et.al.,* 2014) though there are many difficulties in assessing the actual occurrence. Out of total, 40-50% of reproductive males have abnormalities in sperm production such as reduction in quantity, motility or abnormal morphology of sperm (Ferlin, *et.al.,* 2007). Although, the main reason of infertility is still not clear. Autosomal and sex chromosomal abnormalities believed to be 10-15% cause of male infertility (Krausz *et.al.,*2006, Sen *et.al.,* 2013)Y chromosome microdeletions are the leading cause of male infertility (Vineeth, 2011)These microdeletions occur most frequently on the long arm of the Y chromosome (Yq) and all of these are related to the azoospermia factor (AZF) region which contains the genes involved in the growth and development of sperm. This region is located in the deletion intervals 5 and 6 of human Y chromosome and consists of four non – overlapping functional regions called AZFa, AZFb, AZFc and AZFd (Kent *et.al.,* 1999). Each of these regions comprises functionally active genes and transcription units related to spermatogenesis. The most common aberrations that occur in the AZF region are multiple gene deletions in the AZFb and AZFc regions. However, recent studies prove that deletions in all the four regions can cause male infertility (Sakthivel *et. al.,*2009, Mirfakhraie *et.al.,*2010). Microdeletions of AZFa region are proved to be associated with Sertoli cell only syndrome (SCO) syndrome, deletions in AZFb region associated with spermatogenic arrest at the pachytene spermatocyte stage, deletions in AZFc region associated with spermatogenic arrest at the spermatid stage while patients with microdeletions restricted to AZFd region may suffer with mild oligozoospermia or even a normal sperm count associated with abnormal sperm morphology (Calogero *et.al.,*2001)Y chromosome microdeletion indicates the absence of a DNA fragment or gene(s) from the active region of the Y chromosome. Currently, this is believed to be the most frequently occurring structural anomalies of Y chromosome associated with male infertility and STS-PCR technique is considered to be the standard method for the laboratory diagnosis of Y chromosome microdeletions (Suganthi *et.al.,* 2014). The incidence of Y chromosome microdeletions in infertile men varies significantly between studies conducted at different countries, from 0 to 55.56%. The frequency of Y chromosome microdeletions in Indian population ranges from 3 to 29.34% with an average frequency of 8.1% (Suganthi *et.al.,* 2014)

There is paucity of data on Y chromosome microdeletions in the North-East Indian population. The present study was undertaken to determine the frequency of AZF deletions in infertile men with azoospermia, cryptozoospermia, oligozoospermia, asthenozoospermia, teratozoospermia, oligoasthenozoospermia and asthenoteratozoospermia of North East India.

2. MATERIALS AND METHODS

2.1 SELECTION OF SAMPLES/ PATIENTS:

The study population consisted of 500 infertile men compared to 100 normal fertile men seeking andrologic investigation for couple's infertility at fertility clinic. The age of infertile men ranged from 25 to 50 years. They were subjected to detailed clinical and biological investigations, including cytogenic and endrocrinology studies. On the basis of spermiogram, individuals (n=500) were subdivided into 7 groups: azoospermic (0 spermatozoa spz/ml), cryptozoospermic (less than $1x10^6$ spz/ml), oligozoospermic (less than 20×10^6 spz/ml), Asthenozoospermic (less than 32% sperm motility), teratozoospermic (less than 4% morphologically normal sperm), oligoasthenozoospermic (sperm concentration and sperm motility values below the lower reference limit) and asthenoteratozoospermic (lesser sperm motility and morphologically normal sperm count) according to the World Health Organization (WHO). 100 males of proven fertility with normal sperm concentration ($20x10^6$ spz/ml) were selected as control.

2.2 SAMPLE COLLECTION AND DNA EXTRACTION:

Blood and semen samples were collected for DNA extraction. DNA isolation from blood and semen samples was performed using commercially available kit (GeneiPure™). The extracted DNA samples were stored at -20^0C before analysis.

2.3 Y CHROMOSOME MICRODELETION ANALYSIS:

All the subjects were screened for Y chromosome microdeletion by

PCR using oligonucleotide primers as described in the table 1.

2.4 MOLECULAR ANALYSIS

Polymerase Chain Reaction (PCR) was performed according to the standard protocol for analysis of microdeletion in AZF region of the Y chromosome. PCR amplification of the 5 genes/ STS markers was performed using the primer sets as described in table 1. The PCR amplification comprised a total volume of 25µL, which contained 100 to 200 ng of human genomic DNA as template, 2.5 mM dNTP's (2.5mM each of dTTP, dCTP, dGTP, dATP), oligonucleotide primers (0.1 to 2µmol/L each of the forward and reverse primers), 10X Taq DNA polymerase assay buffer (Tris with 15 mM $Mgcl_2$) and 3U Taq DNA polymerase.

The conditions for thermocycling was standardized for the sub-regions, utilizing a TC-512 gradient thermocycler. Samples were subjected to 35 cycles at 94^0C for 30 sec, 53^0C for 45 sec and 72^0C for 60 sec. Initial denaturation was done at 94^0C for 5 min and final extension at 72^0C for 10 mins. The PCR amplified products were separated by electrophoresis on 2% agarose gels and stained with 0.5µg/ml ethidium bromide. Before loading the samples into the wells of the agarose gel, samples were mixed with gel loading dye in the ratio of 1:1. A 100 bp DNA ladder was loaded alongside the products to estimate the band size. A visual record of the gels was made with the help of gel documentation system.

3. RESULTS:-

On analysis of the PCR products in agarose gel the samples where no bands were visible was taken to bear deletion of the particular gene/STS marker (Figure 1).

Among the 500 patients included in our study 123 (24.6%) had azoospermia, 10 (2%) had cryptozoospermia, 103 (20.6%) had oligozoospermia, 112 (22.4%) had asthenozoospermia, 32 (6.4%) were teratozoospermia, 84 (16.8%) had oligoasthenozoospermia and 36 (7.2%) had asthenoteratozoospermia (Figure 2).

In blood of different infertile groups of the present study total Y chromosome deletion was observed as 21 (27.27%) in azoospermia, 5 (6.49%) in cryptozoospermia, 16 (20.78%) in oligozoospermia, 13 (16.88%) in asthenozoospermia, 7 (9.09%) in teratozoospermia, 8 (10.39%) in ologoasthenozoospermia and 7 (9.09%) in asthenoteratozoospermia (Figure 3). Whereas in semen of different infertile groups deletion was detected as 28 (29.79%) in azoospermia, 9 (9.57%) in cryptozoospermia, 17 (18.08%) in oligozoospermia, 10 (12.77%) in asthenozoospermia, 10 (10.64%) in teratozoospermia, 11 (11.70%) in oligoasthenozoospermia and 7 (7.45%) in asthenoteratozoospermia (Figure 4).

The percentage of deletion of different genes /STS markers in different infertile groups are as follows: In sY 84 (AZF a region) of azoospermic males the deletion percentage is higher in blood (57.14%) compared to that of semen (50%), likewise in cryptozoospermic, oligozoospermic and teratozoospermic males also the percentage of deletion was higher in blood as compared to semen i..e.40% in cryptozoospermia (nil in semen), 31.25% in oligozoospermia (11.76% in semen) and 14.29% in teratozoospermia (nil in semen). Whereas in asthenozoospermia, oligoasthenozoospermia and asthenoteratozoospermia the percentage of deletion was higher in semen i..e., 16.67% in asthenozoospermia, 27.27% in oligoasthenozoospermia and 28.57% in asthenoteratozoospermia with no deletion in the blood of the above three groups.

In sY 127 (AZF b region) of azoospermic males the deletion percentage was higher in blood 4.76% (nil in semen), in cryptozoospermic males the deletion percentage was higher in semen 22.22% (nil in blood), in asthenozoospermia the deletion percentage was slightly higher in semen 8.33% (7.69% in blood).Whereas in oligozoospermia, teratozoospermia, oligoasthenozoospermia and asthenoteratozoospermia no deletion was observed in the sY 127 STS marker in both blood and semen. Again in $RBMY_1$ gene there was no deletion in both blood and semen of Cryptozoospermic, teratozoospermic and oligoasthenozoospermic individuals. In azoospermia and oligozoospermia however there was no deletion in blood but deletion percentage in semen found as 3.57% in azoospermia and 5.88% in oligozoospermia. In asthenozoospermia and asthenoteratozoospermia the percentage deletion was higher in blood i.e., 15.38% in asthenozoospermia (8.33% in semen) and 14.28% in asthenoteratozoospermia (nil in semen)

In sY 254 (AZF c region) of azoospermic males the deletion percentage was higher in semen 7.14% (nil in blood), in cryptozoospermic males the deletion percentage was higher in blood 40% as compared to semen i.e 11.11%, in oligozoospermic men the deletion percentage was higher in blood 6.25% but nil in semen. Whereas in asthenozoospermia, teratozoospermia, oligoasthenozoospermia the deletion percentage was higher in semen i.e 25% in asthenozoospermia with no deletion in blood, 20% in teratozoospermia (14.28% in blood) and 18.18% in oligoasthenozoospermia (12.5% in blood) but in asthenoteratozoospermia deletion was higher in blood 42.85% (nil in semen). Again in PRY-2 gene there is no deletion in both blood and semen in Cryptozoospermic individuals. In azoospermic, oligozoospermic and teratozoospermic males their deletion percentage in semen was higher as compared to blood i.e 17.85% in azoospermia (14.28% in blood), 35.29% in oligozoospermia (12.5% in blood) and 40% in teratozoospermia with no deletion in blood. But in asthenozoospermia, oligoasthenozoospermia and asthenoteratozoospermia males the percentage deletion is higher in blood i.e., 38.46% in asthenozoospermia (nil in semen), 25% in oligoasthenozoospermia (9.09 in semen) and 28.57% in asthenoteratozoospermia (nil in semen).

4. DISCUSSION:

The process of gamete formation in males i.e. spermatogenesis is a long and complex process, involving a series of continuous cellular changes which can be divided into three major stages: mitotic proliferation of spermatogonia, meiosis and spermiogenesis. In males, the main causes of impairment of spermatogenesis include anatomical defects, gametogenesis dysfunction, endocrinopathies, immunological problems, ejaculatory failure and environmental toxicity like exposure to environmental pollutants, occupational and lifestyle characteristics.Spermatogenesis is regulated through number of genes on Y chromosome and autosomes. Microdeletions on AZF region of Y chromosome is established as one of the major cause of human male infertility (Simoni, et.al.,2004). Deletions in the AZF region of the Y chromosome directly damage genes in this region that is responsible for the proper course of spermatogenesis (Krausz et.al.,2014)

In male infertility, 15-30% cases involving complex genetic abnormalities can lead to different degree of spermatogenic failure (Massart, et.al.,2012).The relation between male infertility and Y chromosome deletions was first found by Tiepolo et al. (1976).

The incidence of microdeletions in the AZF region has been reported to be 3 to 55 percent in patients with azoospermia (Dabaja et.al.,2013). Although the frequency of microdeletions in azoospermatic patients were different in the literature which is possibly due to ethnic or geographic factors. The most frequent region of the deletions is reported in the AZFc sub region (Behulova, et.al.,2011).Studies had reported varying frequencies ranging from 1.3 to 55% (Osterlund et.al.,, 2000, Foresta et.al., 2001, Chiang et.al., 2004). Many factors could explain this large variation such as sample selection criteria, the STS selection for the research of Yq chromosome microdeletions along with ethnic and geographical location and life style factors.

In our study population, microdeletions were found to be 77/500 (15.4%) in blood and 94/500 (18.8%) in semen of infertile males

VOLUME-6 | ISSUE-4 | APRIL - 2017 • ISSN No 2277 - 8179 | IF : 4.176 | IC Value : 78.46

which is in contrast with the findings of Kdous et al. (2015) where microdeletions were reported as 9.5% in infertile males. The results of the present study are not in conformity with the previous study of Ambulkar et al.(2015) where deletion frequency was reported as 12.8%.Other studies reported high frequencies of microdeletions up to more than 30% deletion in infertile male (Mir et.al., 2006), while some others reported lower frequencies of deletion as low as 4% in comparison to the statistical values reported from all surveys (Zhang, et.al.,2007).Zhang et al. (2015) reported an incidence of microdeletion between 5.1% and 9.6% in the infertile males which do not agree with our findings.

The frequency of microdeletions in the azoospermic group of the present study was 21/77 (27.27%) in blood and 28/94 (29.79%) in semen which is observed to be high as compared to the findings of Kdous et al. (2015) where the frequency of microdeletions in the azoospermic group was just 11.1%. But our findings are in consistent with that of Arundhati et al. (2004) where the frequency of microdeletion was reported to be 29.63%. In the present study the frequency of microdeletions in cryptozoospermic group in blood was 6.49% and 9.57% in semen. The frequency of microdeletions in oligozoospermic group in blood was 20.78% and 18.08% in semen which is higher than that reported by Arundhati et al (2004) and Kdous et al. (2015).

In the present study frequency of microdeletions in cryptozoospermic group in blood was 5/77 (6.49%) and 9/94 (9.57%) in semen. However, this group was not included in the previous studies. Similarly frequency of microdeletions in teratozoospermia and asthenoteratozoospermia in blood and semen was also not reported in the earlier studies. In our study we have found the frequency of microdeletions in blood of teratozoospermic male as 7/77 (9.09%) and 10/94 (10.64%) in semen, likewise the frequency of microdeletions of asthenoteratozoospermia in blood was 7/77 (9.09%) and 7/94 (7.45%) in semen.

The frequency of microdeletion in oligoasthenozoospermia and asthenozoospermia men as reported by Barbhuiya et al. (2013) was 11.1% in oligoasthenozoospermia and 25% in asthenozoospermia, whereas in our study it was observed that the frequency of microdeletion in blood of asthenozoospermia was 13/77 (16.88%) and 12/94 (12.77%) in semen and the frequency of microdeletion in blood of oligoasthenozoospermia was 8/77 (10.39%) and 11/94 (11.70%) in semen.

According to our findings it was observed that the frequency of microdeletion in AZFc region 22/77 (28.57%) is highest, followed by AZFa region 20/77 (25.97%) and AZFb region 5/77 (6.49%) in blood respectively. Similarly in semen also the frequency of microdeletions in AZFc region was found to be highest 26/94 (27.65%) followed by AZFa region i.e.23/94 (24.47%) and then AZFb region i.e. 6/94 (6.38%). Our findings are not in conformity with the previous studies as the most frequent region of deletions was reported in the AZFc region followed by AZFb and AZFa subregion (Boivin, et.al.,2007).In another study it was reported that the frequency of deletions in AZFc region as 10/19 (52.63%), followed by AZFb region which as 6/19 (31.58%) and then by AZFa region as 3/19 (15.79%) (Prafulla, et.al.,2015) which also do not match with our findings.

In our present study we have selected 2 gene/STS marker sY 127 and RBMY1 for AZF b region, 2 gene/STS marker sY254 and BPY-2 for AZF c region but only one gene/STS marker for AZF a region i.e. sY 84 for both blood and semen. It is to be noted that the frequency of microdeletion in AZF a (sY 84) region was higher compared to the frequency of microdeletion of AZF b region using 2 gene/STS marker i.e. sY 127 and RBMY1.

The some amount variation in the number of Y chromosome microdeletion detected in the present study may be attributed to the ethnic diversity and geographical factors of this region.

ACKNOWLEDGEMENT:
This work was supported by fund from DST Govt. of India and the study was conducted at Institutional Biotech Hubs (IBTHubs), Cotton College, Guwahati, Assam sponsored by Department of Biotechnology, Govt. of India.



Figure: 1: Representative Gel photograph showing the PCR analysis using Primer *Pry-2(170bp)*

TABLE 1: PRIMERS USED

Regions /Genes	Markers	Sequences 5' -> 3'	Amplicon size
AZFa	sY84-F	AGA AGG GTC TGA AAG CAG GT	327bp
	sY84-R	GCC TAC TAC CTG GAG GCT TC	
AZFb	sY127-F	GGC TCA CAA ACG AAA AGA AA	274bp
	sY127-R	CTG CAG GCA GTA ATA AGG GA	
AZFb	RBMY1-F	CTT TGA AAA CAA TTC CTT TTC C	900bp
	RBMY1-R	TGC ACT TCA GAG ATA CGG	
AZFc	sY254-F	GGG TGT TAC CAG AAG GCA AA	400bp
	sY254-R	GAA CCG TAT CTA CCA AAG CAG C	
AZFc	PRY-2-F	AAG AAG AGG AGC ACA CCA	170bp
	PRY-2-R	ATA TGC CTA CTT AAA AAA TGT T	

TABLE 2: Number of deletion in AZF a, b, c region in different infertile groups

Type of Infertility		AZF a	AZF b		AZF c	
		SY-84	SY-127	$RBMY_1$	SY-254	PRY-2
Azoospermia	B	12(57.14%)	1(4.76%)	0(0%)	0(0%)	3(17.85%)
	S	14(50%)	0 (0%)	1(3.57%)	2(7.14%)	5(14.28%)
Cryptozoospermia	B	2 (40%)	0(0%)	0(0%)	2(40%)	0(0%)
	S	0 (0%)	2(22.22%)	0(0%)	1(11.11%)	0(0%)
Oligozoospermia	B	5 (31.25%)	0(0%)	0(0%)	1(6.25%)	2(35.29%)
	S	2 (11.76%)	0(0%)	1(5.88%)	0(0%)	6(12.5%)
Asthenozoospermia	B	0 (0%)	1(7.69%)	2(15.38%)	0(0%)	5(38.46%)
	S	2 (16.67%)	1 (8.33%)	1 (8.33%)	3(25%)	0(0%)
Teratozoospermia	B	1 (14.29%)	0(0%)	0(0%)	1(20%)	0(0%)
	S	0 (0%)	0(0%)	0(0%)	2(14.28%)	4 (40%)
Oligoasthenozoospermia	B	0 (0%)	0(0%)	0(0%)	1(18.18%)	2(25%)
	S	3 (27.27%)	0(0%)	0(0%)	2(12.5%)	1(9.09%)
Asthenoteratozoospermia	B	0 (0%)	0(0%)	1(14.28%)	3(42.85%)	2(28.57%)
	S	2 (28.57%)	0(0%)	0(0%)	0(0%)	0(0%)

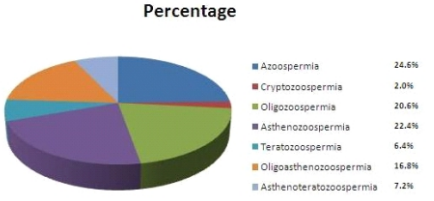


FIGURE: 2 Percentage of patients with different types of Infertility

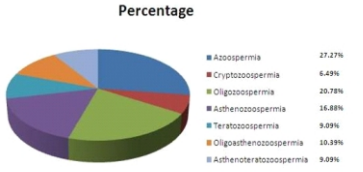


Figure: 3 Percentage of deletions in blood of different types of Infertility

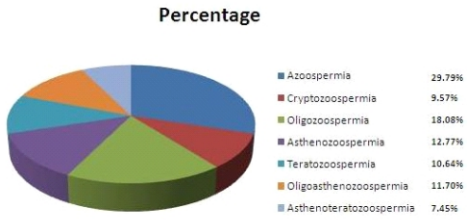


Figure: 4 Percentage of deletions in semen of different types of Infertility

REFERENCES:

1. Arundhati SA, Prochi FM, Nandkishor J N, Dattatray JN, Smita SG, Suresh BD, Vijay MB, Mahadev TK , Firuza RP ,(2004), A Study of Y Chromosome Microdeletions in Infertile Indian Males, Int J Hum Genet, 4(3): 179-185.
2. Behulova R, Varga I, Strhakova L, Bozikova A, Gabrikova D, Boronova I, Repiska V,(2011), Incidence of microdeletions in the AZF region of the Y chromosome in Slovak patients with azoospermia, Biomed Pap Med Fac Uni Palacky Olamouc Czech Repub, , 155(1): 33-38.
3. Bhasin S, de Kretser DM, Baker HW, (1994) Clinical review 64: pathophysiology and natural history of male infertility, J Clin Endocrinol Metab, 79:1525–9.
4. Boivin J, Bunting L, Collins JA, Nygren KG,(2007) International estimates of infertility prevalence and treating-seeking: potential need and demand for infertility medical care, Hum Reprod, 22:1506-1512.
5. Calogero AE, Garofalo MR, Barone N, De Palma A,(2001), Spontaneous regression over time of the germinal epithelium in a Y chromosome-microdeleted patient: Case report. Hum. Reprod, 16: 1845-1848.
6. Chandley AC, Cooke HJ, (1994) Human male fertility- Y-linked genes and spermatogenesis. Hum Mol Genet, 3:1449–52.
7. Chiang HS, Yeh SD, Wu CC, Huang BC, Tsai HJ, Fang CL, (2004) Clinical and pathological correlation of the microdeletion of Y chromosome for the 30 patients with azoospermia and severe oligoasthenospermia, Asian J Androl, 6:369-375.
8. Dabaja AA, Schlegel PN, (2013)Microdissection testicular sperm extraction: An update. Asian J Androl, 15(1): 35-39.
9. Ferlin A, Arredi B, Foresta C, Genetic causes of male infertility, Reprod Toxicol, 2007, 22:133-141.
10. Foresta C, Moro E, Ferlin A, (2001)Y chromosome microdeletions and alterations of spermatogenesis, Endocrine Rev, 22: 226-239.
11. Foresta C, Ferlin A, Moro E, (2001) Y chromosome microdeletions and alterations of spermatogenesis, Endocr Rev, 22:226–39.
12. Kent- First M, Muallem A, Shultz J, Pryor, Roberts K, Nolten W, Meisner L, Chandley A, Gouchy G, Jorgensen L, Havighurst T and Grosch J,(1999) Defining regions of the Y AZF region (AZFd) by Y-chromosome microdeletion detection, Mol Reprod Dev, 53:27-41.
13. Khabour OF, Fararjeh AFS and Alfaouri AA,(2014) Genetic screening for AZF Y chromosome microdeletions in Jordanian azoospermic infertile men, Int J Mol Epidemiol Genet, 5(1):47-50.
14. Krausz C, Degl'Innocenti S, Y chromosome and male infertility: update Front Biosci, (2006), 11:3049-3061.
15. Krausz C, Hoefsloot L, Simoni M, Tuttelmann F, (2014) European Academy of Andrology, European Molecular Genetics Quality Network, EAA/EMQN best practice guidelines for molecular diagnosis of Y chromosomal microdeletions. State of the art 2013. Andrology, 2(1): 5-19.
16. Massart A, Lissens W, Tournaye H, Stouffs K, (2012) Genetic causes of spermatogenic failure, Asian J Androl, 14:40-48.
17. Mir DO, Saied S, Mortaza B, Kiarash Attar, (2006) Y Chromosome Microdeletions in Idiopathic Infertile Men from West Azarbaijan, Urology Journal, 3(1): 38-43.
18. Mirfakhraie R, Mirzajani F, Kalantar SM, Montazeri M, Salsabili N, Pourmand GR and Houshmand M, (2010)High prevalence of AZFb microdeletion in Iranian patients with idiopathic non-obstructive azoospermia, Indian J Med Res, 132: 265-270.
19. Moez K, Fethi Z, Amel Z, (2015)Cyotgenetic and Y Chromosome Microdeletions Screening in Tunisian Infertile Men. Donnish Journal of Genetics and Molecular Biology, 1(1): 1-5.
20. Osterlund C, Sergersteen E, Aver S, Pousette A,(2000) Low number of Y chromosome deletions in infertile azoospermic men at a Swedich Andrology Centre, Int J Androl, 23: 225-229.
21. Prafulla A, Ajay C, Jwalant W, Aaditya T, Asoke P,(2015)Prevalence of Y chromosome Microdeletions in Idiopathic Azoospermia Cases in Central Indian Men, Journal of Clinical and Diagnostic Research, 9: GC01- GC04.
22. Sakthivel PJ, Gopenath TS and Swaminathan M, (2009) Genetics of human male infertility, Singapore Med J, 50:336-347.
23. Sen S, Pasi AR, Dada R, Shamsi MB, Modi D,(2013) Y chromosome microdeletions in infertile men: prevalence, phenotypes and screening markers for the Indian population, J Assist Reprod Genet, 30:413-422.
24. Simoni M, Bakker E, Krausz C, (2004,)EAA/EMQN best practice guidelines for molecular diagnosis of y-chromosomal microdeletions: State of the art, Int J Androl, 27:240-49.
25. Suganthi R, Vijesh VV, Nambiar V and Benazir JAF, (2014)Y chromosomal microdeletion screening in the workup of male infertility and its current status in India, Int J Fertil Steril, , 7(4):253-266.
26. Vineeth VS and Malini SS, (2011)A Journey on Y Chromosomal Genes and Male Infertility, Int J Hum Genet, 11(4): 203-215.
27. Zhang F, Lu C, Li Z, Xie P, Xia Y, Zhu X, (2007,)Partial deletions are associated with an increased risk of complete deletion in AZFc: a new insight into the role of partial AZFc deletions in male infertility, J Med Genet, 44:437-444.