

ABSTRACT

Infertility is the major concern among the married couples and in at least 50% of them; the male partner is the sole contributor to the problem. Majority of these men are diagnosed with no aetiology and hence they are idiopathic cases. The current study was planned to evaluate the role of genetic and molecular factors in 200 men with idiopathic infertility. The work involved cytogenetic and Y chromosome micro-deletion analysis. Patients with cytogenetic and Yq micro-deletion anomalies were excluded for further studies. Further, infertile cases were evaluated for seminal reactive oxygen species by chemiluminescence assay. Oligo-asthenozoospermic (OA) patients were screened for sperm mtDNA *ATPase* and *NADH* gene mutations. One hundred infertile men with sperm count less than 20 million/ml were screened for sperm nuclear protein gene nucleotide alterations. Sperm DNA integrity was analysed by sperm chromatin structure assay (SCSA). Semen analysis was carried out as per WHO (1999) guidelines with slight modification. Patient's detailed history, physical findings and serum reproductive hormones were recorded in a predesigned proforma. Lifestyle habits such as smoking, alcohol consumption, and cell phone usage were graded as moderate and severe and recorded in the proforma.

Out of 200 infertile men screened, 13% harbored cytogenetic abnormalities. This frequency is higher when compared to the previous report from Indian population. Azoospermia factor (AZF) microdeletions were detected in 9.5% infertile men, where AZFc deletion was predominant (6%) followed by AZFb (2%) and AZFa (1.5%) deletions. This is in accordance to the range reported in the world wide population. Level of ROS in both washed and neat semen was found to be significantly ($P < 0.0001$) higher in infertile men compared to controls. However, no correlation between ROS level and the sperm parameters was observed. The ROS levels in raw semen showed a strong positive correlation ($r = 0.842$, $p < 0.0001$) with ROS levels in washed semen.

Significantly higher frequency of nucleotide changes in mitochondrial *ATPase6*, *ND4* and *ND5* gene was observed in infertile OA men, who had significantly ($P < 0.001$) higher ROS levels, compared controls. Majority of the mitochondrial nucleotide changes observed were found to be pathogenic by polyphen computational analysis.

A strong negative correlation ($r = -0.260$) between testosterone level and body mass index (BMI) was observed in infertile men. Smoking, alcohol and cell phone usage had moderate effect on sperm parameters and serum prolactin level in smokers and alcoholics was found to be significantly ($p < 0.01$) lower

compared to non smoker and non-alcoholic men. Infertile men using cell phone for longer duration had significantly ($p<0.05$) lower sperm motility compared to non-users or moderate users.

The average mean DNA fragmentation index (DFI) in infertile men was found to be 40.85% which was significantly higher ($p<0.0001$) compared to average mean DFI (25.62%) of control men. DFI cut off value of 30.28% between infertile and control men with 88.1% sensitivity and 93.9% specificity was obtained. A strong negative correlation between DFI and sperm parameters and a positive correlation ($r= -0.330$, $p=0.03$) with duration of infertility was observed. Sperm nuclear protein gene mutation screening revealed no significant difference in the frequency between infertile and controls. Sperm samples with severe asthenozoospermia revealed several ultrastructural changes.

In conclusion, screening for cytogenetic and Yq anomalies are highly recommended in patients opting for ARTs as there is increased incidence of these anomalies in Indian population. Smoking, alcohol, cell phone usage have moderate to severe detrimental effect on male fertility. Therefore, infertile men may be advised to quit smoking and alcohol to improve their sperm parameters. Increased BMI has strong association with decreased serum testosterone levels. Sperm DFI cut off level of 30.42% may be useful in better management of infertile Indian men as conventional semen analysis is a moderate predictor of fertility potential.

Oxidative stress contributes majority of the pathology in idiopathic infertile men and measurement of ROS in neat/raw semen is easy, fast and reliable compared to washed semen. Hence, early detection of OS and suitable antioxidants therapy may prevent further deterioration of semen quality. Increased mitochondrial DNA mutations in OA men may have better diagnostic and prognostic value in ART, as there is no paternal transmission of paternal mtDNA. It is also important to distinguish the genetic cause of male infertility, whether it is nuclear or mitochondrial, to counsel such couples prior to undergoing assisted reproduction

Key words: Male infertility; Chromosome abnormalities; Y microdeletions; Reactive oxygen species; mtDNA mutation; sperm DNA integrity.