Infertility is the inability of a sexually active, non-contraception couple to achieve pregnancy in one year. Generally, infertility is classified as either primary (67-71%) when no pregnancy has ever occurred or secondary (29-33%) where there has been a pregnancy, regardless of the outcome. It affects 13-18% of couples of reproductive age worldwide and is treatable in many cases [1]. Although, infertility is not life threatening, it is psychologically a traumatic condition associated with social stigma in certain societies resulting in intense social and parental pressure to attain biological parenthood; hence many infertile couples choose to undergo assisted reproductive technology as a form of treatment.

In recent years, it has been demonstrated that up to 50% of infertility cases are associated with male factor because of spermatogenic failure; interestingly in which ~70% of the underlying etiological cause(s) are known but remaining 30% are referred to as idiopathic infertility [2]. Thus, the etiology of male infertility is complex and can be reflected in a variety of conditions like defective spermatogenesis, congenital absence of bilateral vas deferens (CABVD), infections, and immunological disorders such as antisperm-antibodies, diabetes, obesity, pituitary disorders, and imbalance in levels of gonadotrophic hormones. Genetic factors are considered to be significant in male infertility [3]. Hence, the aim of the study is to investigate the chromosomal aberrations, azoospermia factor (AZF), cystic fibrosis transmembrane regulator (CFTR) gene mutations in the peripheral blood lymphocytes (PBL) of infertile men with abnormal sperm parameter and compare it with healthy fertile control subjects to delineate the genetic causes of male infertility.

This thesis comprises of six chapters. The first chapter summarizes about male factor infertility: incidence, classification, etiology, genetic factors, and treatment options. In around 50% of infertile couples, male-related causes are involved, such as impaired spermatogenesis and decreased sperm count, due to non-genetic as well as genetic causes. Genetic factors contributing to male reproductive failure are often multiple and the relation between them are
inconsistent; varying from a single gene defects, interactions of multiple genes and chromosomal abnormalities. Extensive analysis of literature were reviewed and presented to derive the objectives of the study.

Second chapter describes in detail about the materials and methods applied to study chromosomal abnormalities, Yq microdeletions and CFTR gene polymorphisms associated with male factor infertility. The study protocol was approved by the Institutional Ethics Committee with Ref No: (SRMC/RP/4505). The study population consists of infertile men \((n=175)\) and controls \((n=110)\); the mean age of the controls and cases were 39.5±0.4 and 37.5±0.4 years respectively. Average duration of infertility is of 8.5±0.5 years and infertile subjects are classified into azoospermia (AZ) as 84.6%, oligoasthenoteratozoospermia (OAT) as 13.7%; severe oligoasthenoteratozoospermia (SOAT) as 1.1% and 0.6% of globozoospermia as per patient’s record. About 2ml of lithium heparinized and 2ml of \(\text{Na}_2\)-EDTA PBL was collected in sterile vacutainers with informed consent from the subjects visiting the Department of Obstetrics and Gynecology, Sri Ramachandra Medical Center, Porur, Chennai. About 25 metaphases from each sample were eye-karyotyped; two metaphases were captured with Image Analyzer, (Cytovision Software Version 4.0), and interpreted as per International System for Cytogenetic Nomenclature guidelines [4].

To study microdeletions on chromosome Y and M470V polymorphism of CFTR gene, genomic DNA was isolated as per the manufacturer’s protocol (Bioserve-DNA Isolation Kit). The quantity of the DNA was checked with spectrophotometer and the quality was checked with 0.8% agarose gel electrophoresis. Microdeletions were detected by performing Sequence-Tagged Site (STS) PCR based techniques on controls and cases’ peripheral blood genomic DNA. All STS PCR primer sequences were obtained from published literature [5] and AZF regions studied were \(AZFa\-sY\ 84\ (326\ bp), sY 86\ (320\ bp), AZFb\-sY\ 127\ (274\ bp), sY134\ (301\ bp)\) and \(AZFc\- sY\ 254\ (400\ bp), sY 255\ (126\ bp)\). The PCR product was separated using agarose gel electrophoresis (2%) and observed under UV-transilluminator; presence or absence, a band
of expected size (base pair) for all the six loci were investigated with the above-mentioned controls and cases and was documented. Fertile male and female samples were used as positive and negative controls.

A missense polymorphism M470V of CFTR gene on exon 10 was amplified, digested by \textit{HphI} restriction enzyme was studied with PCR-RFLP. The amplified PCR product showed a band size corresponding to 491 bp. Restriction digestion of PCR product showed either M/M homozygous (wild type allele at 491 bp), V/V homozygous (mutant allele at 300 and 191 bp) or M/V heterozygous (restriction sites at 491, 300 and 191 bp) respectively. The allele and genotype frequencies between the control and cases were compared for statistical significance using SPSS software version 16.0.

The third chapter describes about the results of cytogenetics obtained from GTG banded chromosomes in study subjects. Chromosomal analysis of control subjects showed normal karyotype of 46,XY without any structural or numerical abnormalities. However, in infertile cases 86.32% showed a normal karyotype of 46,XY and remaining 13.68% with different types of chromosomal abnormalities (CA). Among the azoospermia subjects, 2.85% showed an abnormal karyotype: 47,XXY (non-mosaic) Klinefelters and 46,X,del(Y)(q11.2), deletion on Y chromosome. A 0.57% of OAT cases showed [45,XY,t(13;14)(q10;10)] Robertsonian translocation. Thus, overall 3.42% infertile subjects displayed constitutional chromosomal abnormalities (associated with both autosomes and sex chromosomes); 6.84% of variants and 3.42% with low-level mosaicism. The impact of CA in male infertility was found to be high and inversely related to sperm count. The obtained results were compared with the published reports.

It has been proposed that the existence of a key factor, which controls spermatogenesis encoded by a gene that is localized within the euchromatic region of the Y chromosome long arm (Yq11) which was called as the “Azoospermia factor” [6] and many genes controlling spermatogenesis
are mapped within these AZF regions. Microdeletions of these genes are associated with divergent testicular histological profiles, ranging from Sertoli cell-only syndrome (SCOS), hypospermatogenesis (HS) to spermatogenic arrest (SGA). Therefore, in addition to GTG karyotyping, Y chromosome microdeletion was carried out in the study subjects and the obtained results are given in fourth chapter. The frequency of microdeletions was found to be 9.14% in the azoospermic and 3.42% in the oligoasthenoteratozoospermic infertile men. An overall 12.56% of infertile subjects showed microdeletions in one or more sequence-tagged sites (STS) with a percentage of 1.14, 2.28, 9.14 for AZFa (sY84, sY86), AZFb (sY127, sY134) and AZFc (sY254, sY255) respectively. However, control subjects did not show any deletion in all the loci.

The fifth chapter discusses about CFTR polymorphisms associated with male infertility obtained by PCR-RFLP. Polymorphisms in CFTR gene, has been observed in 1–2% of male infertility cases contributing to azoospermia [7]. Hence, the allele and genotype frequency of M470V polymorphism in the exon-10 of CFTR gene, in controls (n=100) and infertile men (n=157) was investigated. In the control subjects analyzed, the frequency of M and V alleles was found to be 0.55 and 0.45; the distribution of the M/M genotype was 36%, M/V genotype 18% and V/V genotype 46%. Whereas, in infertile men the frequency of M/M, M/V and V/V genotypes are 43%, 71% and 43% respectively. The frequency of M and V alleles was found to be 0.5 each and the alleles were in Hardy-Weinberg equilibrium. The obtained results showed that M/V genotype was found to be significantly higher in cases than controls (OR 3.3; 95% CI 1.67-6.52 P< 0.001) suggesting an association of CFTR gene M470V polymorphism for infertility.

The sixth chapter discusses about the correlation of CA, Yq microdeletion, M470V polymorphism and male infertility. The obtained results (i) among the infertile men showed 3.57% of both CA and Yq microdeletion; (ii) 3.89% of CA and MV heterozygotic alleles and (iii) 15.05% of Yq microdeletion and MV heterozygosis in CFTR gene. Only 2.02% cases showed CA, Yq microdeletion and M470V polymorphism. A substantial number of infertile men, however,
present with a history associated with fertility problems and have normal findings with genetic testing. However, the availability of novel whole genome approaches encourages future studies, and will likely contribute to the identification of de novo and recurrent genetic factors.

References


