ESTABLISHMENT OF A MOLECULAR DIAGNOSTIC SYSTEM FOR DETECTING Y-CHOROMOSOMAL MICRODELETIONS WHICH CAUSE MALE INFERTILITY

A PROJECT REPORT PRESENTED BY

KARIHARAN THIRUCHELVAM

to the Board of Study in Biochemistry and Molecular Biology of the

POSTGRADUATE INSTITUTE OF SCIENCE

In partial fulfillment of the requirement
for the award of the degree of

MASTER OF SCIENCE IN EXPERIMENTAL BIOTECHNOLOGY

of the

UNIVERSITY OF PERADENIYA

SRI LANKA

2006

ABSTRACT

ESTABLISHMENT OF A MOLECULAR DIAGNOSTIC SYSTEM FOR DETECTING Y-CHROMOSOMAL MICRODELETIONS WHICH CAUSE MALE INFERTILITY

KARIHARAN THIRUCHELVAM

Postgraduate Institute of Science
University of Peradeniya
Peradeniya
Sri Lanka

Male infertility is mainly caused by severe spermatogenic impairment and is governed by genetic and non-genetic factors. The Azoospermic factor region (AZF) located at the long arm (Yq-euchromatin) of the Y chromosome harbors crucial candidate genes for spermatogenesis and its control. Microdeletions in this region cause severe spermatogenic defects such as azoospermia and oligospermia and result in male infertility. The main objective of this study was to establish a molecular diagnostic system for detecting Y-Chromosomal microdeletions which cause male infertility and assess the microdeletion pattern (type and frequency) of infertile men in Sri Lanka.

Peripheral venous blood samples were obtained from 62 infertile men who were azoospermic (34), oligoseprmic (7), severe oligospermic (18) and normospermic (3) patients from infertility clinics (Vindana Institute-Colombo and The Reproductive Biology Lab - Faculty of Medicine, University of Ruhuna). Genomic DNA was extracted from blood leucocytes by using Promega genomic DNA isolation kit. Six non-polymorphic, single copy STS markers were selected from AZF loci - AZFa (sY84, sY86), AZFb (sY127, sY134), and AZFc (sY254, sY255) according to EMQN/EAA suggestion and adjacent STS primers were grouped into two sets of multiplex master mixes, set A and B. The Multiplex PCR assay developed in this study amplified specific STS from 3 sub regions (a,b,c) of the AZF loci and PCR products were analyzed by gel electrophoresis. Failure of any STS amplification indicated microdeletion in the AZF locus. Appropriate internal (X specific ZFX and Y specific SRY) and external (positive fertile male DNA, negative female DNA and negative water blank) PCR amplification quality controls were set in each PCR reaction to validate the results.

Deletions were detected in 9 infertile men (14.5%) out of 62. No deletions were observed in fertile men and normospermic men. Deletions were only observed in 7 azoospermic (20.6%) and 2 severe oligospermic (11%) patients. Single deletion in the AZFc region had the highest frequency (n=4, 44.4%), followed by less AZFb (n=2, 22.2%) and no single deletion were observed in AZFa (n=0, 0%). Combined deletions were detected in 2 patients who were azoospermic ·AZFb+c (n=1, 11.1%) and AZFa+b+c (n=1, 11.1%). Single and combined deletions of AZFa and b loci resulted in azoospermia but single AZFc deletion resulted severe oligospermia and azoospermia in similar proportion and AZFc combined with a (AZFa+b+c) and b (AZFb+c) resulted in azoospermia.

The Low cost molecular diagnostic system developed in this study for the first time in Sri Lanka effectively detected microedeletions in the AZF region at the long arm of the Y chromosome and analyze the deletion pattern in a group of infertile men in Sri Lanka. The deletion pattern was consistent with previous studies in other regions. This system provides an affordable, cost effective routine screening method with high degree of accuracy, sensitivity, rapidity and specificity for low sperm count infertile men (TESE/ICSI candidates) at infertility clinics and sperm donors at sperm banks. Inheritance of infertility to the male offspring can be avoided by this screening and well informative genetic counseling can also be provided for better therapeutic treatment option. This diagnostic system can be used to analyze microdeletion in the large infertile male population in Sri Lanka to assess the accurate deletion pattern and to establish refined deletion map to identify and characterize novel spermatogenic candidate genes and may lead to predisposition studies of Yq deletions by analyzing haplotypic variation of AZF deleted Y genome to determine predisposed haplotype groups in Sri Lankan infertile male population.