

PCR-based detection of Y-chromosomal microdeletions in a selected group of infertile males

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Male infertility is commonly associated with severe spermatogenic impairment. The Azoospermic factor region (AZF) located at the long arm (Yq-euchromatin) of the Y chromosome harbors crucial candidate genes in its sub regions AZFa,b,c for spermatogenesis and its control. In the last decade microdeletions in these sub regions have been shown to be associated with the spermatogenic defects of azoospermia and oligospermia. The objective of this study was to establish a molecular diagnostic system for detecting Y-Chromosomal microdeletions and to assess the microdeletion pattern (type and frequency) in a selected sample of infertile Sri Lankan males.

DNA was extracted from venous blood samples of 62 infertile men who were azoospermic (34), oligoseprmic (7), severe oligospermic (18) and normospermic (3) patients from infertility clinics. Six non-polymorphic, single copy STS markers were selected from the AZF region – AZFa (sY84, sY86), AZFb(sY127, sY134), and AZFc (sY254, sY255). Adjacent STS primers were grouped into two sets of multiplex PCR master mixes, set A and B. PCR products were size fractionated by agarose gel electrophoresis with ethidium bromide staining. Two internal controls and three external controls were applied to control for false negatives, specificity, sensitivity, DNA contaminations and reagent contaminations.

Deletions were detected in 9 infertile men (14.5%) out of 62. No deletions were observed in fertile men and normospermic infertile men. Deletions were observed in 7 (20.6%) of the 34 azoospermic patients and 2 (11%) of the 18 severe oligospermic patients. Single deletions in the AZFc region had the highest frequency (n=4, 44.4%), followed by AZFb (n=2, 22.2%). Combined deletions were detected in 2 patients who were azoospermic AZFb+c (n=1, 11.1%) and AZFa+b+c(n=1, 11.1%). In this study, single and combined deletions of AZFa and b loci were associated with azoospermia and single AZFc deletions were associated with severe oligospermia and azoospermia in similar

proportions and AZFc combined with a (AZFa+b+c) and b (AZFb+c) were associated with azoospermia. The molecular diagnostic system developed in this study for the first time in Sri Lanka, effectively detected deletion patterns similar to previous studies in other geographic locations.

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