mixing for one minute then 300 ul from protein lysis solution was added with another mixing. The samples were then centrifuged, the supernatants were collected in a clean tubes and the DNA precipitated with equal volume of isopropanol alcohol. DNA samples were pelleted by centrifugation, washed with 70% ethanol alcohol, air dried and re-suspended with 100 ul of distilled water.

The DNA concentration and purity were checked.The agarose gel electrophoresis was also adopted to confirm the presence and integrity of the extracted DNA.

PCR Assay:

Six primers supplied by alpha DNA company-Canada were used in PCR to determine the presence of Y chromosome microdeletions in AZFa, AZFb and AZFc locuses. The primers sequences were shown in Table 1.

Table 1: Primers sequences and products.

STS	Left primer	Right primer	AZF interval	Products in pb
SY84	5-GTGACACACAGACTATGCTTC-3	5-ACACACAGAGGGACAACCCT-3	AZFa	320
SY127	5-GGCTCACAAACGAAAAGAAA-3	5-CTGCAGGCAGTAATAAGGGA-3	AZFb	274
SY254	5-GGGTGTTACCAGAAGGCAAA-3	5-GAACCGTATCTACCAAAGCAGC-3	AZFc	400

PCR was performed according to (19) using a thermal DNA cycler machine (Tec gene-UK). Cinagene PCR Kit (Iran) was utilized. A hundred nano grams (ng) of denaturated DNA and 40 picomole from each primer were added to the PCR master mixture. The reaction was initiated in a volume of 50 ul. A total of 20 cycles of polymerization was carried out. Ten micro liter from each amplified DNA , 0.2 ug of lambda Hind III+EcoR1 fragments as a marker were mixed with 2 ul of loading buffer and electrophoresed through a 1% agarose gel for 30 minutes at 50 Hz volts. The gel was then stained, visualized under UV light and photographed.

Results

Screening of 25 azoospermic men with the sequence tagged sites- STS-markers specific to AZF regions showed deletion in 6 individuals (Figures 1, 2 and 3) which accounts for 24% of the total azoospermic men analyzed. Of 6 individuals with AZF deletions, deletion of the AZFc region alone was detected in 2 individuals which accounted for 33.3% of the total individuals (Table 2). One azoospermic man showed deletion in the AZFb region (16.7%) and 3 azoospermic men showed deletions in the AZFa +AZFc regions (50%).None of the control men showed deletion for STS markers.

The cytogenetic analysis revealed morphologically normal Y chromosome in all examined samples.