are neutral which do not affect the fertility status and phenotype of the individual [16, 17].

The current study aimed to detect chromosomal abnormalities and Y microdeletions (AZF deletions) among a number of azoospermic men.

Materials and Methods

Semen and blood were collected from the azoospermic patients- aged between 20 to 51 years- who attended the infertility unit in the Institute of embryo research and infertility treatment/Al-Kadhimya from September 2006 to October 2007.

Semen sampling and analysis:

Using the 1999 WHO guidelines [18] a semen sample from each subject was collected into a clean, dry and sterile vial after abstinent of 3-4 days. After incubation at 37.5C for 30 minutes, the semen samples were centrifuged at 2500 rpm for 10 minutes and the pellets were examined under light microscope.

The azoospermia was defined as no sperm was present in the semen.

Blood Collection:

Five ml from peripheral blood was collected from 25 azoospermic men and four controls (one female +three fertile man).Each blood sample was divided into two aliquots, one aliquot was added to heparinized tube for cytological examination, the other aliquot was added to EDTA tube for DNA extraction.

The EDTA blood samples were centrifuged at 2000 rpm for 10 minutes. The serum of each blood sample was collected in a clean and sterile tube and used for further assays. The WBC layer from each sample was collected in a sterile tube and used in DNA extraction.

Blood Culture:

A half milliter from each heprinized blood sample was cultured in 5 ml of standard supplemented RPMI 1640 medium containing 20% fetal calf serum and 2% of phytohemagglutinin (PHA) (prepared by the molecular biology Department\Iraqi center for cancer and medical genetic research-ICCMGR-Baghdad-Iraq) in a sterile tubes. The tubes were cultured at 37°C for 72 hours. A hundred micro liter of cholchicine (0.45 mg\ml) was added to each culture. After 20 minutes, the cells from all culture tubes were harvested by centrifugation (2000rpm\10 mins).The supernatants were discarded and the cells redissolved with the remaining solution. The cells were exposed to mild hypotonic treatment with 3ml of 0.075 M KCL at 4°C.The cells was precipitated by another centrifugation. The supernatants were discarded, cells redissolved with remaining hypotonic solution and fixed with 5 ml fixative solution (3 methanol: 1 Glacial acetic acid).Centrifugation and fixation were repeated four times at intervals of 20 minutes. Slides were stained the following day for 10 minutes in 10 ml 5% buffered Giemsa solution, pH 6.8. Three slides were prepared for each sample and 50 metaphases were examined from each sample for chromosomal abnormalities.

DNA extraction:

The WBC layers collected from the EDTA blood samples were used in DNA extraction.

The DNA was extracted according to the Wizard genomic DNA purification kit (Progema/USA).One third milliliter from the WBC suspension was mixed with 900 ul of cell lysis buffer. Samples were incubated at 20°C for 10 minutes .The nuclei were pelleted by centrifuging at 3000 rpm for 10 minutes.The supernatant was discarded and the pellet redissolved with the remaining solution. Three hundred micro liter from nuclei lysis buffer was added to the nuclei suspension with gentle