

## **Centeromeric breakage and fragile site expression in cryptorchidism - A case report.**

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### **Abstract**

The present case was phenotypically male except that testes were not present in the scrotum. Hence in this case of "cryptorchidism", the genetic study becomes imperative for thorough analysis. Chromosomal study revealed more than 13.3 % centromeric breaks per cell in metaphase. The high incidence (3.6%) of common fragile sites expression (7q11, 2q21, 3p14) were also noticed after addition of 5-azacytidine. These "genetic factors" might influence the weight of the testis or alter hormonal level which is essential for normal growth and descent of testis in mammals.

**Key words:** 5- Azacytidine, Crytorchidism, Fragile sites, Centromeric breakage.

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### **Introduction**

In human, Y-chromosome consists of both euchromatin and heterochromatin [1] The chromosome science plays a significant role in diagnostic as well as in basic research. Many factors like increased intra abdominal pressure, hormonal secretion and differentiated growth of the organs between gubernaculum & the body wall are required for descent of the testis. The studies based on genetic aspects are still scanty. Fragile - sites are non random distributed loci on chromosomes which are prone to form breaks/gaps at metaphase stage of cell-division under specific culture conditions that either inhibit DNA replication or facilitate repair mechanism. These fragile sites are structural defective regions because of close association to proto-oncogene [2]. The studies of "common fragile sites" are very difficult because of low frequency of metaphase present in cell population [1,3]. In the present case of "cryptorchidism", the cytogenetic features were evaluated with the aim to understand the mechanism of abnormal sexual development (ASD) at molecular level.

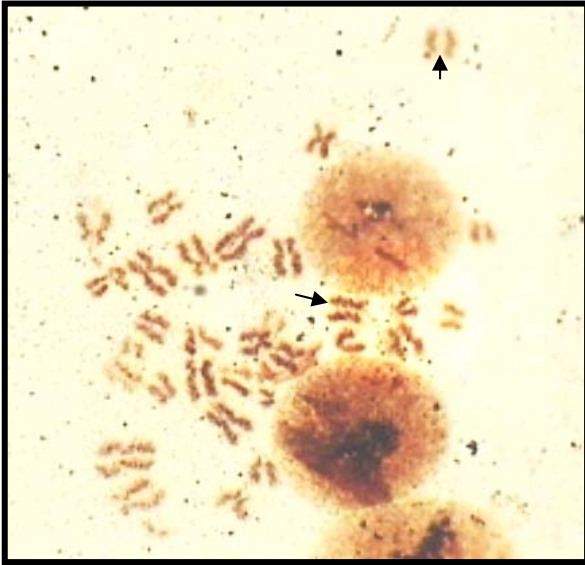
### **Materials and Methods**

The proband aged three and half year showing normal male phenotypes and well developed scrotal pouch without testis referred to cytogenetic laboratory for evaluation. The mother was 34 year old while father was 40 years old at the time of birth of proband. The delivery was full term and normal. The peripheral heparinized whole blood sample (0.5ml) was collected both from proband and father as

a control after written consent under sterile conditions for short term lymphocytes culture. The lymphocytes were grown in complete culture media (RPMI-1640) having 5 % FBS with or without supplement of folic acid (1µg/ml), L-glutamine (0.5µg/ml), phytohemagglutinin -M and antibiotics for 72 hours at 37°C. 5 -Azacytidine (4 µgm/ml), DNA inhibitors was added to the cultures before 7 hours to the termination of the cultures to observe fragile sites expression in present study. Colchicine (0.01mg/ml) as mitotic (inhibitor) arresting agent was added to culture 3 hour before harvesting. Chromosome preparations were made according to routine procedure. Cells were fixed in pre-chilled mixture of acetic acid & methanol (1:3). Slides were prepared, GTG banding using trypsin and visualized by 5% Giemsa as well as AgNOR stain with standard protocol [4,5].

### **Results**

Interestingly, cytogenetics study reveals that the frequency of centromeric breakage (13.3 %) was observed in proband as documented in metaphase spread stained by silver staining as documented in figure-1 while father karyotype showed normal 46, XY picture using GTG bandings technique. Another aspect of study reveals significant variable frequency of total structural chromosomal alteration when compared with control ( $p < 0.05$ ) using *chi square test* including chromatid / chromosome breaks and fragile sites. The frequency of such breaks varies from 3.3 % to 6.6 % were observed in normal culture condition and after addition of folic acid 7q11 (6.6%)



**Figure 1:** Metaphase spread showing centromeric breakage using highly sensitive silver staining for visualization and characterization of heterochromatic region of chromosomes (as indicated by arrow head)

and 5-azacytidine 2q21, 3p14 (3.3%) into lymphocyte cultures of the same patient.

## Discussion

Normally descent of testis start to develop in the lumbar region during fetal life and it reaches to iliac fossa by the end of the third month of prenatal life. The centromeric regions are mainly consists of constitutive heterochromatin and normally the Y- chromosome is almost heterochromatized and plays significant role during differentiation and further development of male gonads [6]. However, the exact role of such regions (centromeric region) during the development and differentiation of testis has not been defined including descent of testis. The dysmorphology of chromosome including high incidences of fragile sites induced by 5-azacytidine in the present study is due to DNA methylation [7] because 5-azacytidine is DNA methyltransferase (DNMT) inhibitor. From the present study it is still not clear that which isoform of the DNMT is facilitated with *de novo* methylation of enzymes in embryonic development [8] hence further studies are required.

Interestingly, the present study reveals high incident of "common fragile sites" (7q11, 2q21, 3p14) just contrast to the study of Richard et al [9] what they commonly found in low proportion i.e. less than 5% of metaphase preparation. Authors believe that the higher incidence genetic damage is probably due to habitual food / nutritional habit (sensitivity) or differential sensitivity to fragile sites

(2q21, 3p14). It has also been reported that in Down syndrome patients, 5-AZC induces significant changes including centromeric breakage because "fragility" of chromosomes exist at/ near to centromeric regions [10, 11,12].

Authors have hypothesized that in the present case, (cryptorchidism) the arrest of descent of testis may take place either within the abdominal cavity, or in inguinal canal or between the superficial inguinal ring and the scrotum, has not been defined by the ultrasound findings in the present case.

Undoubtedly, hormonal factors including teratogen like cyclophosphamide also play a significant role for undescents of testis [13,14]. However, these *de-novo* structural abnormalities (centromeric breakage & fragile sites) which altered the entire heterochromatin and a part of euchromatin region are associated with the sterility of males [15].

In human chromosome, centromere is the important component because these regions consist of constitutive heterochromatin and highly repeated satellite DNA sequences. Segregation of centromeric regions is normally seen during cell-propagation at telomeric ends which help to stabilize movement of chromosomes during cell-division. Earlier report suggested that the genetic causes of undescended testis are due to multiple crossing overs including involvement of heterochromatin region and structural changes of Y-chromosomes [16]. Because of heterochromatic nature of Y-chromosome it is also quite possible that normal differentiation and further development of male gonads are influenced by such genetic alterations. In the present study authors have reported first time such cytogenetics findings and hypothesized that specific sequences (palindromes) on the centromeric region might have responsible for cryptorchidism [17]. Interestingly, the present study has important implications on the assessment of potential risk factor either nutrition deficient diet or environmental including unknown factors present in the populations. Although, further study is required to collect more data with large sample size to make this study significant at both cellular as well as molecular level in Indian population.

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