Cytogenetic analysis of prostate carcinoma patients

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Abstract
Background & objectives: Prostate carcinoma is the second leading cause of cancer death in men above age of 60 years worldwide. More than 80% of the cancers concomitantly arise with Benign prostate hyperplasia. Prostate carcinoma is associated with various lower urinary tract symptoms, which affect their day to day life. The aim of the present investigation was to find out the major chromosomal aberrations present in Prostate carcinoma patients and to make a comparison with other study.

Methods: In present study 05 cases of prostate carcinoma were taken on the basis of clinical diagnosis from the Gujarat Cancer Research Institute (GCRI), Ahmedabad, Gujarat, India, during the period of March 2011 to October 2011. The patients were analyzed for chromosomal aberrations using cultured peripheral blood lymphocytes with their pre-informed written consent.

Results: In the present study, all 5 cases were > 60 years of age. 3(60%) cases of prostate carcinoma have elevated PSA level (>4 ng/ml). In adenocarcinoma of prostate gain, deletion, and translocation of 7q22-q31 is common. In present study, Karyotype analysis reveal 01 prostate carcinoma patient had 46,XY,del(7)(q31). In present study 2 cases shows evidence of metastasis.

Conclusion: Prostate carcinoma is found in > 60 years of age. Chromosome 1, 2, 3, 6, 7, 9, 16, 22, X and many more chromosomes were affected in prostate carcinoma patients, Common chromosomal aberration is 46,XY,del(7)(q31). Patients with prostate carcinoma have elevated PSA level (>4 ng/ml). Prostate carcinoma associated with metastasis have poor prognosis.

Keywords: AUASI score, Chromosomal aberration, Karyotype, Prostate carcinoma, PSA

1. Introduction
Prostatic carcinoma is a disease in which cells in the prostate gland become abnormal and start to grow uncontrollably, forming tumours which usually originate in the Peripheral zone of the prostate gland.

Prostate carcinoma is the most commonly diagnosed malignancy in men over the age of 60 years. Above this age, the incidence and mortality rate increase exponentially. Prostate specific antigen (PSA) level increases with age. Patients with prostate carcinoma produce larger amounts of PSA.

American urological association symptom index (AUASI) score is self-administered questionnaires, used to assess the severity of three storage symptoms (frequency, nocturia, urgency) and four voiding symptoms (feeling of incomplete
emptying, intermittency, straining, and a weak stream). How frequently the patient experiences each symptom is rated on a scale of 1 to 5.

Various treatment modalities for prostate carcinoma include open or minimal invasive surgery e.g., Trans urethral resection of prostate (TURP), Trans urethral needle ablation (TUNA) etc., medication like alpha-blocker, hormonal therapy. Among these modalities of treatment surgical management may affect sexual functions of individual.

The frequency of chromosome instability in peripheral blood lymphocytes is generally indicative of increased cancer risk for those exposed to DNA damaging agents. Deletions, translocations, inversions and mosaics were the major chromosomal aberrations observed in prostate carcinoma. Chromosome 1, 2, 3, 6, 7, 9, 16, 22, X and many more chromosomes were affected in prostate carcinoma patients.\(^1\)

This study of clinical & Karyotypic profile of patients with prostate carcinoma has been helpful to find out chromosomal abnormalities & genetic cause of prostate carcinoma so that proper management and genetic counselling can be done.

2. Material and Method

For the present study, 05 clinically diagnosed Prostate carcinoma patients were selected from the Gujarat Cancer Research Institute (GCRI), Ahmedabad, Gujarat, India, during the period of March 2011 to October 2011. Their detailed clinical history & clinical examination finding were noted. About 2 ml of venous blood was collected in sodium heparinized vacutte from each patient after their pre-informed written consent.

Culture setting was done on the same day of sample collection in Genetic laboratory, Anatomy department, B. J. Medical college, Ahmedabad, using freshly tapped blood with MEM media, Foetal Bovine Serum and PHA (phytohemagglutinin) and put it in incubator at 37°C. After an incubation period of 69½ hours, metaphase was arrested by adding colchicine. After total 72 hours of incubation the lymphocytes were harvested by centrifusing cells to remove culture medium (3000 rpm for 10 minute) & addition of hypotonic solution (KCI 0.075 M) at 37°C for 20 min to swell the cells, and treated thrice with chilled Carnoy’s fixative (3:1 ratio of methanol : acetic acid) and finally the metaphases on the slides were obtained. Then those slides showing metaphases with good morphology were selected and kept under dry wooden boxes for aging process.

After 7 days, GTG banding procedure was done using freshly prepared Trypsin-EDTA solution and Giemsa stain.\(^3\) About 25 metaphase plates were observed in each case and finally, a photograph was obtained from a good quality metaphase slide with the help of a Leica’s automatic karyotyping machine (100x). The chromosomal findings were described according to the International system of Human Cytogenetic Nomenclatures and finally, karyotypes were prepared using Leica’s Automatic Karyotyping software.

3. Result

In the present study 05 clinically diagnosed prostate carcinoma patients were studied for cytogenetic assessment by conventional Karyotyping. All 05 patients were above 60 years of age. Out of all the cases 1 case had a positive family history of prostate carcinoma. As per AUASI score assessment all 5 patients had severe (20-35) score.

Following observation were found on Prostate specific antigen (PSA) level findings. Out of 05 cases, 02 patients had normal PSA level while 03 patients had elevated PSA level. [Table-1]

<table>
<thead>
<tr>
<th>PSA Level</th>
<th>Normal (≤4ng/ml)</th>
<th>Increased</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>02</td>
<td>03</td>
<td>05</td>
</tr>
</tbody>
</table>

In present study out of 05 patients studied; 04 (80%) patients involve peripheral zone of prostate, while 1 (20%) prostate cancer patient have involvement of central zone of prostate. [Table-2]
Table-2: Zone of prostate affected in patients studied

<table>
<thead>
<tr>
<th>Zone of Prostate</th>
<th>Peripheral Zone</th>
<th>Central Zone</th>
<th>Transition Zone</th>
<th>Anterior Fibro-muscular Zone</th>
<th>Total No. of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>04</td>
<td>01</td>
<td>00</td>
<td>00</td>
<td>05</td>
</tr>
</tbody>
</table>

Out of 05 Prostate cancer patients studied; 02 (40%) Prostate cancer patients show presence of metastasis, while 03 (60%) Prostate cancer patients show no evidence of metastasis. [Table-3]

Table-3: Associated with metastasis in patients studied:

<table>
<thead>
<tr>
<th>Metastasis</th>
<th>Present</th>
<th>Absent</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Prostate cancer patients</td>
<td>02</td>
<td>03</td>
<td>05</td>
</tr>
</tbody>
</table>

In our conventional karyotype analysis we found that only one 61 years old patient had 46,XY,del(7)(q31).[Image-1] While 03 cases had normal Karyotype and in rest 1 cases metaphase was not found. [Table-4]

Table-4: Cytogenetic findings by Conventional Karyotyping in patients studied.

<table>
<thead>
<tr>
<th>Metaphase Finding</th>
<th>Numerical Abnormality</th>
<th>Structural abnormality (deletion)</th>
<th>Normal</th>
<th>Metaphase not found</th>
<th>Total No. of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>0</td>
<td>01</td>
<td>03</td>
<td>01</td>
<td>05</td>
</tr>
</tbody>
</table>

Image-1: Karyotype showing 46 XY,del(7)(q31)

4. Discussion

Prostate carcinoma is the second leading cause of cancer death in men above age of 60 years worldwide. Evidence suggests that the PSA level increases with age. Patients with prostate carcinoma produce larger amounts of PSA. Muneer M. S. Sharaf et al., found that out of 78 patients, 5 (6.4 %) have low level (0 to 2.5 ng/ml), 2 (2.6 %) have moderately elevated level (10-19.9 ng/ml), 50 (64.1 %) have highly elevated level (≥ 20 ng/ml) of PSA. Sushma T.A., found that out of 65 cases serum PSA values over 10ng/ml were seen in 10 benign (16.6%) cases and 3 malignant (60%) cases. V. Balachandar et al., in 2007, noted in his study that out of total 45 prostate cancer patients, PSA level of collected blood samples was ≤ 10.0 ng/ml in 39 subjects (86.7%) and ≥10.0 ng/ml in 6 subjects (13.3%). V. Balachandar et al., in 2008, found that range of PSA values in Prostate Cancer patients were 8 to 12 ng/ml. In present study out of 05 patients, 02 patients had normal PSA level while 03 patients had elevated PSA level (>4ng/ml).

In more than 70 % Prostate cancer is originated from the Peripheral zone of prostate gland. 25 % of prostate cancer may arise from the central zone. Zhao F.J. et al., noted in their study that there are morphological and ultra-structural differences in stromal cells from transitional zone (TZ) and peripheral zone (PZ) of the normal prostate. In all, 514 differentially expressed genes were selected by microarray analysis; 483 genes were more highly expressed in stromal cells.
from TZ and 31 were more highly expressed in those from PZ. Co-culture with PZ stromal cells and transforming growth factor-beta1 (TGF-beta1) increased the tumour growth of PC-3 cells in vitro and in vivo, as well as Bcl-2 expression. Ultrastructures and gene expression differ between the stromal cells from TZ or PZ of the normal prostate, and stroma-epithelium interactions from TZ or PZ might be responsible for the distinct zonal localization of prostate tumour formation. In present study out of 05 patients studied; 04 (80%) patients involve peripheral zone of prostate, while 1 (20%) prostate cancer patient have involvement of central zone of prostate.

Most common metastasis associated with prostate cancer is skeletal metastasis spread through venous route. Lukas Bubendorf MD et al, 8 noted in his study that among 19,316 routine autopsies performed from 1967 to 1995 on men older than 40 years of age, the reports from those 1,589 (8.2%) with prostate cancer were analyzed. Hematogeneous metastases were present in 35% of 1,589 patients with prostate cancer, with most frequent involvement being bone (90%), lung (46%), liver (25%), pleura (21%), and adrenals (13%). Maximum frequency of spine involvement occurred in smaller tumours (4 to 6 cm) as compared with the maximum spread to lung (6 to 8 cm) and liver (> 8 cm), suggesting that spine metastases precede lung and liver metastases in many prostate cancers. Surveillance, Epidemiology, and End Results (SEER) cancer statistics, 9 shows that from 2001-2007, 82% prostate cancer were localized (confined to primary site), 11% were regional (spread to regional lymphnodes), 4% were distant (cancer has metastasized), 3% were unknown (unstaged). In the present study out of 5 prostate cancer patients; 2 patients shows evidence of metastasis.

N. B. Atkin and Marion C. Baker, 10 studied four carcinomas and one leiomyosarcoma of the prostate, in which a deleted chromosome 10, del (10) (q24), was found in all four carcinomas. Three of the carcinomas also had a deleted chromosome 7, del(7)(q22), while the fourth had a 7p+. Lundgren R. et al, 11 noted that out of 57 patients with prostate cancer, normal karyotypes were found in 24 tumours, structural nonclonal aberrations were detected in 18 and clonal karyotypic abnormalities in 15 tumours. Chromosomes 1, 7, and 10 were most frequently affected. Miyauchi T et al, 12 found that out of 10 cases of adenocarcinoma of the prostate, structural analysis disclosed abnormality of chromosome 16 in four Prostate carcinoma, deletion of Y chromosome seen in three prostate carcinoma, abnormality of chromosomes 7, 14, 15, 18, and 19 in two prostate carcinoma, and abnormality of chromosomes 3, 4, 17, and 21 in one prostate carcinoma. Satoru Takahashi et al, 13 reported that numeric changes of chromosomes 7, 8, X, and Y are common in prostate carcinoma. H Matsuura et al, 14 found in his study that out of 28 prostate cancers, the most common aberration in prostate cancers was a gain of chromosome 8 (57%), with numerical aberration of chromosome 7 being the second most frequent anomaly (50%). Robert B. Jenkins et al, 15 studied 25 prostate specimens, of which six tumours had no apparent anomaly for any chromosome 7 probe. Nine tumours showed apparent simple gain of a whole chromosome 7, whereas one tumour had apparent simple loss of a whole chromosome 7. Four tumours had gain of the chromosome 7 centromere and additional over-representation of the 7q-arm. One tumour had over-representation of 7q31 without any apparent anomaly of the chromosome 7 centromere, and one tumour had apparent loss of the chromosome 7centromere with no apparent anomaly of the 7q-arm. Three tumours had gain of the chromosome 7 centromere and loss of the 7q31 region. Their data indicate that the 7q-arm, particularly the 7q31 region, is genetically unstable in prostate cancer. A. R. Brothman et al,16 in 1999, reported that loss of the Y chromosome, gain of 7, 8, and X, and interstitial deletions on 6q, 7q, 8p, 10q, 13q, 16q, 17q, and 18q are the most prevalent chromosomal aberration in prostate cancer. Strehmeyer D. M. et al, 17 noted in his study that the most frequent DNA copy number gains were on chromosome 3, 4, 7, 8, 10, 11, 12, 13, and X. The most frequent losses were on chromosomes 2, 5, 6, 8, 10, 13, 15, and 16. V. Balachandar et al, 6 in 2007, found that among 45 blood samples from prostate cancer patients, major chromosomal aberrations like deletion, translocation, inversion seen in chromosomes 1, 3, 5, 6, 7, 9, 13, 16, 18 and X and mosaic were identified in patients. V. Balachandar et al, 1 in 2008, studied 18 prostate cancer patients and found that chromosome 1, 2, 3, 6, 7, 9, 16, 22, and X were the affected chromosome in Prostate Cancer patients.

In present study we found that only one 61 years old patient had 46,XY,del(7)(q31). While 03 cases had normal Karyotype and in rest 1 case metaphase was not found.

5. Conclusion

Identification of chromosome aberrations facilitates cloning of the relevant genes that may be involved in prostate carcinoma. The present study shows that the chromosome aberrations may be a potential biomarker for prostate carcinoma. These markers may have relevance in diagnosis and staging of prostate carcinoma, and thus may reduce the need for invasive testing. This may help to establish the basis to augment our ability to counsel person on the recurrence risk with greater accuracy. However the cases that are found normal karyotypes by conventional cytogenetics, require to be
confirmed by more specific molecular genetic studies like Fluorescence in Situ Hybridization (FISH) technique, to exclude any molecular level anomaly.

References


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