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Identification of Chromosome Aberrations among Benign Prostatic Hyperplasia Patients in Tamilnadu, Southern India

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ABSTRACT 27 blood samples were collected from various hospitals to study the chromosomal aberrations (CA) in benign prostatic hyperplasia patients of Tamilnadu, Southern India. Equal number of normal healthy control blood samples has been collected by using questionnaire. After signing a consent form, volunteers provided blood sample (5 ml) to establish cell cultures at 52 h. For CA analysis, 100 metaphases from each subject were evaluated. Major chromosomal aberration like deletion, translocation, inversion and mosaic have been observed in experimental subjects. These types of CA were frequently observed in chromosomes 1, 6, 8, 13, 16, 18. In control, very low levels of major CA were observed compared to experimental subjects Identification of chromosome alterations may be helpful in devising better therapeutic strategies.

INTRODUCTION

Clinical benign prostatic hyperplasia (BPH) is a common disease and occurs in about one quarter of men in their fifties, one third of men in their sixties, and about half of all men ≥80 years (McVary 2006). A causative role for inflammation in the pathogenesis of BPH was first proposed in 1937 (Moore 1937). However, for the major part of the 20th century, the embryonal reawakening theory (McNeal 1990) dominated the field of BPH.

In India, the annual estimate of cancer for the year 2001 was 0.98 million and the annual mortality in 2000 was 0.7 million (Greenlee 2001). It is relatively rare for prostate cancer to be diagnosed in men below 50 years of age, but above this age, the incidence and mortality rates increase exponentially (Haas and Sakr 1997).

The frequency of chromosome instability in peripheral blood lymphocytes is relevant biomarker for cancer risk in humans, reflecting early biological effects of genotoxic carcinogens and individual cancer susceptibility (Hagmar et al. 1998; Bonassi et al. 2000). An increased frequency of chromosome aberrations in circulating lymphocytes is generally considered indicative of increased cancer risk for those exposed to DNA damaging agents (Bonassi et al. 1995).

Genetic instability often can be detected at the chromosomal level, resulting in loss or gain of whole chromosomes or portions thereof. However, earlier detection and high resolution discovery of instability would allow for specific treatment of each tumor. Genetic alterations drive tumor progression toward heterogeneity and diversity in prostate cancer (Kim et al. 2000; Chaib et al. 2003; Strohmeyer et al. 2004). One of the diversities in this gland is hyperplastisity of epithelial cells, which appears in both prostate cancer and benign prostatic hyperplasia (BPH). There are several reports of similarities between prostate cancer and BPH. More than 80% of the cancers concomitantly arise with BPH in prostates (Bostwick et al. 1992). Some molecular changes in hyperplastic cells are similar to those seen in cancer (Mascoska et al. 1994; Phillips et al. 1994).

In spite of the available reports in literature very little is known about the spontaneous background levels of chromosomal alterations in the peripheral blood lymphocytes of Benign prostatic hyperplasia patients. The aim of the present investigation was to find out the chromosomal aberrations present in BPH patients of Tamilnadu.

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MATERIALS AND METHODS

Subject Recruitment

Five ml of blood sample was collected from each of 27 BPH patients in various hospitals of Tamilnadu state, who did not undergo any treatment such as hormonal therapy, chemotherapy and radiation therapy. The subjects were recruited in the age group of 40 – 80 yrs; they were divided into Group I(40-60 yrs) and Group II(61 -80 yrs).In both BPH patients and controls 21 (77.8%) subjects were included in Group I and 6 (22.2%) subjects were included in Group II. The PSA level of collected blood samples was d" 10.0 mg/ml (86.6 %) in 23 subjects and ≥ 10.0 mg/ml (13.3%) in 4 subjects. Healthy controls were selected in the same area. All controls had prostate-specific antigen (PSA) levels < 4.0 mg/ml (90% had PSA levels and < 2.0 mg/ml in 10%) along with confirmation by digital rectal examination. Data on medical and family history of cancer, smoking habits, and occupational history were obtained through an interviewer-administered questionnaire as well as from review of the patient's hospital records of both control and diseased patients.

CHROMOSOME ABERRATION ASSAY

All chemical reagents were purchased from Sigma Chemicals, except colcemid that was obtained from Gibco Laboratory. Blood samples were set up to establish leukocyte cultures following standard procedures in our laboratory (Hoyos et al. 1996). 0.5 ml blood was added to 4.5 ml RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM l-glutamine, 1% streptomycin-penicillin, 0.2 ml reagent grade phytohemagglutinin, and was incubated at 37 ºC. After 50 hr, cultures were treated with 0.1 g/ml colcemid to block cells in mitosis. Lymphocytes were harvested after 52 hr by centrifuging cells to remove culture medium (800–1000 rpm), addition of hypotonic solution (KCl 0.075 M) at 37 ºC for 20 min to swell the cells, and treated twice with Carnoy's fixative (3:1 ratio of methanol:acetic acid). Slides were carefully dried on a hot plate (56 ºC, 2 min). Three days later, slides were stained using the trypsin-giemsa technique. For the CA analysis, 100 metaphases in first cell cycle were evaluated per subject under a microscope (100×) to identify numerical and structural CA. Observations were recorded on master tables and later transferred to a computer file.

RESULTS

A total of 54 subjects corresponding to 27 BPH Patients and 27 normal healthy individuals were recruited for this study. The BPH patients had not undergone prior treatment including hormonal, chemotherapy, or radiation therapy. The subjects were recruited with age between 40 and 80 yrs. In table 1, the CA frequently exhibited were in chromosomes 1, 6, 8, 13, 16, 18 (Fig.1-4). Major chromosomal aberrations are shown in Table 3. Interestingly, controls displayed very low level of major CA as shown in table 2 and Figure 5. In BPH mean ± S.D values were Group I 1.47 ± 0.51, Group II 2.83 ± 1.83 and In Control Group I 0.14 ± 0.35; Group II 1.5 ± 0.83. Observations were found to be statistically significant ANOVA (P>0.05) for BPH patients.

DISCUSSION

Cancer is a consequence of genetic or epigenetic alterations in a variety of genes that are fundamental to the process of growth, cell proliferation, differentiation and programmed cell death (Sandberg 1991). Each alteration, whether an initiating or a progression-associated event, may be mediated through gross chromosomal change and hence has the potential to be detected cytogenetically (Solomon et al. 1991). The relationship between presence of high frequencies of chromosome aberrations and predisposition to cancer has been established in syndromes. An increased frequency of chromo-some aberrations in circulating lymphocytes is general-

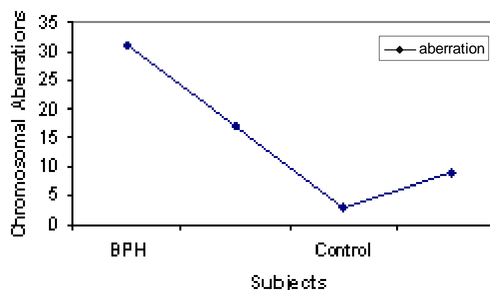

Fig. 1. Age wise increase of CA in BPH patients compared to controls.

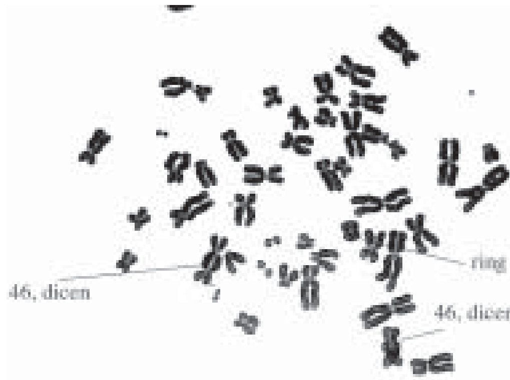

Fig. 2. Ring chromosome and dicentric chromosome

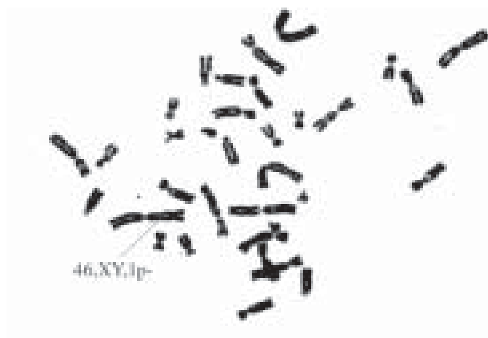

Fig. 3. Chromatid gap in short arm of 1st chromosome (46, XY, 1p-)

Fig. 4. Satellite formation in 13th chromosome - 46, XY, 13s+

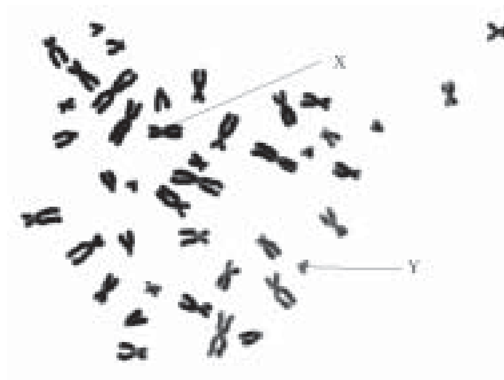

Fig. 5. Normal male (Control) - 46, XY

ly considered indicative of increased cancer risk for those exposed to DNA damaging agents. Bonassi et al. (1995) reported a significant increase in the mortality ratio for all cancers in subjects who had shown increased levels of chromosomal aberrations in their lymphocytes. Essentially, the data from both these studies when pooled indicated that the frequency of chromosome instability in peripheral blood lymphocytes is a relevant biomarker for cancer risks in humans, reflecting early biological effects of genotoxic carcinogens and individual cancer susceptibility (Hagmar et al. 1998; Bonassi et al. 2000). Chromosomal instability has been described in many human dysplastic lesions and is considered a primary event in neoplastic transformation as well as a marker of progression to cancer (Rabinovitch et al. 1999; Burt et al. 2000; Hawkins et al. 2000).

In the present study subjects were recruited with age group of 40 – 80 yrs (Fig.1) because one quarter of men in their fifties, one third of men in their sixties, and about half of all men were of e”80 years (McVary 2006).

Incidence of BPH is increasing, partly because of aging population and partly because of sporadic screening using measurements of PSA (Brothman et al. 1994; Steýlen et al. 1994). In the present study, samples were selected on the basis of PSA level.

Although some population’s differences may be attributable to differences in diet and life style, there is strong evidence that genetic alterations, both somatic and heritable, play a major role in prostate cancer etiology (Schaid et al. 1998)

In the present investigation, major chromosomal aberrations were frequently displayed in

Table 1: Frequency of chromosomal aberrations present in BPH patients.

S.No	*Particulars Case No. (n = 45)*	*Age (years)*	*Chromosomal abnormalities identified by G-banding*				*Total*
			Deletion	*Translocation*	*Inversion*	*Mosaic*	
1	BPH001	45	1	-	-	-	1
2	BPH002	52	-	1	-	-	1
3	BPH 003	57	2	-	1	-	3
4	BPH 004	48	-	1	-	-	1
5	BPH 005	49	-	-	-	-	-
6	BPH 006	57	2	1	-	-	3
7	BPH 007	62	-	1	-	-	1
8	BPH 008	68	-	1	-	2	3
9	BPH 009	41	1	-	-	1	2
10	BPH 010	49	-	1	-	-	1
11	BPH011	59	3	-	-	1	4
12	BPH012	71	3	2	-	-	5
13	BPH013	48	-	-	-	-	-
14	BPH014	53	1	-	-	-	1
15	BPH015	58	-	-	1	-	1
16	BPH016	58	1	-	-	-	1
17	BPH017	60	2	-	-	1	3
18	BPH018	62	-	1	-	-	1
19	BPH019	66	1	1	-	-	2
20	BPH020	43	-	-	-	-	-
21	BPH021	55	1	-	-	-	1
22	BPH022	51	-	1	-	1	2
23	BPH023	48	-	-	-	1	1
24	BPH024	55	1	-	1	-	2
25	BPH025	77	3	2	-	-	5
26	BPH026	60	-	2	-	-	2
27	BPH027	55	1	-	-	-	1

Table 2: Frequency of chromosomal aberrations present in controls.

S.No	*Particulars Case No. (n = 45)*	*Age (years)*	*Chromosomal abnormalities identified by G-banding*				*Total*
			Deletion	*Translocation*	*Inversion*	*Mosaic*	
1	BPH001	45	1	-	-	-	1
2	BPH002	52	-	1	-	-	1
3	BPH 003	57	2	-	1	-	3
4	BPH 004	48	-	1	-	-	1
5	BPH 005	49	-	-	-	-	-
6	BPH 006	57	2	1	-	-	3
7	BPH 007	62	-	1	-	-	1
8	BPH 008	68	-	1	-	2	3
9	BPH 009	41	1	-	-	1	2
10	BPH 010	49	-	1	-	-	1
11	BPH011	59	3	-	-	1	4
12	BPH012	71	3	2	-	-	5
13	BPH013	48	-	-	-	-	-
14	BPH014	53	1	-	-	-	1
15	BPH015	58	-	-	1	-	1
16	BPH016	58	1	-	-	-	1
17	BPH017	60	2	-	-	1	3
18	BPH018	62	-	1	-	-	1
19	BPH019	66	1	1	-	-	2
20	BPH020	43	-	-	-	-	-
21	BPH021	55	1	-	-	-	1
22	BPH022	51	-	1	-	1	2
23	BPH023	48	-	-	-	1	1
24	BPH024	55	1	-	1	-	2
25	BPH025	77	3	2	-	-	5
26	BPH026	60	-	2	-	-	2
27	BPH027	55	1	-	-	-	1

Group I (40-60 yrs); Group II (61-80 yrs).

Table 3: Major chromosomal aberrations in BPH patients.

S. No.	*Particulars (Case No.)*	*Age Group (Yrs)*	*Chromosomal complements*
1	BPHP 0101	Group I (35-45 years)	46, XY, inv (16)
2	BPHP 0106	Group I (35-45 years)	46, XY, / 46 XY,del (6p-)
3	BPHP 0108	Group I (35-45 years)	46, XY, inv (7)
4	BPHP 0115	Group I (35-45 years)	46 XY, del ($1q^-$)
5	BPHP 0117	Group I (35-45 years)	46 XY, del ($6p^-$)
6	BPHP 0121	Group II (46-55 years)	46, XY, t (6q;16q+)
7	BPHP 0124	Group II (46-55 years)	46, XY, inv (9)
8	BPHP 0126	Group II (46-55 years)	46 XY, del ($5p^-$)
9	BPHP 0130	Group II (46-55 years)	46 XY, del ($8q^-$)
10	BPHP 0133	Group III (56 years and above)	46, XY, t (6q;16q+)
11	BPHP 0135	Group III (56 years and above)	46, XY, /46,XY del (5p-)
12	BPHP 0137	Group III (56 years and above)	46 XY, del (Yq^-)
13	BPHP 0139	Group III (56 years and above)	46 XY, del ($1q^-$)

chromosomes 1, 6, 8, 13, 16, 18. In control, very low levels of major CA such as deletion, translocation, mosaic and inversion were identified compared to experimental subjects.

In the present study, Chromosome 1 showed the deletion and translocation in BPH patients. Chromosome 1 has a breakage-prone site, which has been reported to be sensitive to environmental clastogens (Conforti-Froes et al. 1997) and is thought to be involved in both early and late stages of tumor development (Grosovsky et al. 1996). Several reports suggest that chromosome 1 is involved in prostate cancer whether through the presence of prostate cancer susceptibility genes or through the disruption of common pathways involved in cancer development.

In our study, chromosome 6 showed deletions, translocation and mosaics in BPH patients. Almost a third of prostate cancer cases showed LOH as a consequence of deletion of the long arm of chromosome 6, particularly 6q14-21 (Cooney et al. 1996; Srikantan et al. 1999). The frequency of 6q deletion in invasive prostate cancer was fivefold higher than in organ-confined prostate cancer (Srikantan et al. 1999).

The earliest cytogenetic studies identified an 8p23 deletion in hormone-unresponsive sublines of LNCaP cells (Konig et al. 1989). In chromosome 8 deletion and translocations were observed, so also in the case of chromosome 16 in CaP patients. Chromosome 13 showed deletion and inversion in CaP patients. LOH involving the long arm of chromosome 13 has been reported in as many as a third of prostate cancers (Cooney et al. 1996; Li et al. 1998). An increased rate of LOH was initially identified in chromosome 16q (Kunimi et al. 1991). Structural abnormalities and trisomies of chromosome 16 in prostate cancer were also identified through karyotyping (Miyauchi et al. 1992).

The present study showed CA like deletion, translocation in Chromosome 18. Loss of chromosome 18 was frequently observed in SV40-transformed human prostate cancer cell lines. Deletion of the long arm of chromosome 18 was observed in over 40% of primary prostate cancer (Kunimi et al. 1991; Ueda et al. 1997).

In conclusion, in this pilot study, we showed that chromosomal instability in peripheral blood lymphocytes may be a potential biomarker for benign prostatic hyperplasia susceptibility. To date, the molecular genetic events associated with the initiation and progression of benign prostatic hyperplasia remain poorly understood. It has long been considered that genetic instability plays a pivotal role in the development and progression of human cancer (Loeb 1991), and as with most types of human cancer, multiple genetic changes are probably necessary for prostate carcinogenesis.

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