

## Chromosomal Instability in Peripheral Blood Leucocytes of Oesophageal Cancer Patients

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**KEY WORDS** Peripheral leucocytes; genetic instability; specific chromosomes; oesophageal cancer

**ABSTRACT** Aim of present study was to study the chromosomal instability in peripheral blood leucocytes of oesophageal cancer patients. Purpose of this study was to assess whether aberrations in leucocytes occur at random or involve specific chromosomes. Blood samples of 10 oesophageal cancer patients (preoperative) and 10 age and sex matched controls were collected from various hospitals at Amritsar (Punjab, India). Leucocytes were cultured using standard leucocyte culturing technique and metaphases were analysed for chromosomal aberrations. A variety of chromosomal aberrations including chromosome gaps, chromatid gaps, acentric fragments, acrocentric associations, terminal deletion, hypodiploidy, polyploidy, double minutes, Robertsonian translocation i.e. t (D&G), loss of chromosome 2, 7q-, 10, 11, 12, 15, 17, 19, 21,Y, and gain of 3, 4, 10, 19, and 22 chromosome were seen in peripheral blood leucocytes of oesophageal cancer patients. No such aberrations were seen in controls samples. High frequency of aberrations involving specific chromosomes in peripheral blood leucocytes similar to those reported in tumor tissue indicates that chromosomal instability is probably constitutional in nature and participates in cancer predisposition.

### INTRODUCTION

All the tumors contain genetic alterations including change in DNA sequence as well as cytogenetically visible changes such as chromosome loss, gain, and translocations. Genetic instability has been hypothesized as a cardinal feature of cancer and can be of two types: Microsatellite instability (MIN) and Chromosomal instability (CIN). Chromosomal instability in cancer is a natural side effect of the malignant transformation process driven by preceding mutation in growth controlling

oncogenes and tumor suppressor genes. An increased rate of chromosomal mutations results in an elevated frequency of other genetic abnormalities, which probably directly promotes tumor cell proliferation and viability (Cahill et al. 1999). Chromosomal instability is not confined to the tumor tissue only but has been reported in other tissues like peripheral blood leucocytes in retinoblastoma (Czeizel et al. 1974; Takabayashi et al. 1983; Nunez et al. 1984), breast cancer (Madhavi et al. 1990), colorectal cancer (Richard et al. 1994), renal cell carcinoma (Wang et al. 1982), skin cancers (Nordenson et al. 1984) and in skin fibroblasts in Multiple Endocrine Neoplasia-1 (Scappaticci et al. 1991). The previous cytogenetic studies in leucocytes of oesophageal cancer patients have reported fragile sites 1p13-36, 4q21-q31, endoreduplication and tetraploid cells (Wu 1991), high-level amplification on 2p24-33, 6q21.1-q14, 7q11.2-31, 8q22-24.1, 13q2.2-34 and 8q13-qter and loss of 8p, Xp, and gain on chromosome 5p (Du Plessis et al. 1999).

The frequency of oesophageal cancer is high in North India. In the present study chromosomal instability in peripheral blood leucocytes was studied in the oesophageal cancer patients from areas adjoining Amritsar city of Punjab state, India. Purpose of this study was to assess whether the chromosomal aberrations in leucocytes occur at random or involve specific chromosomes only.

### MATERIAL AND METHODS

After clinical examination, blood samples of 10 oesophageal cancer patients (7 males and 3 females) were collected prior to operation from the surgical wards of various hospitals at Amritsar according to the guidelines of ethical committee constituted by Guru Nanak Dev University, Amritsar. Relevant informations including symptoms, duration of the disease,

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habits, habitat, occupation, and exposure to mutagens were recorded on predesigned questionnaire. The leucocytes of patients and 10 age and sex matched controls (7 males and 3 females) were cultured in RPMI 1640 medium according to standard culturing technique given by Moorhead et al. (1960) with some modifications. Slides were banded according to Benn and Perle method (1986). Banded slides were scanned for numerical and structural chromosomal aberrations

### RESULTS

8 patients (6 males and 2 females) were rural and belonged to low socioeconomic group. 6 male patients had agriculture as their main occupation and had an exposure to pesticides and chemical fertilizers, one male patient worked in textile mill. Two females patients belonged to family of agriculturist and were housewives. Out of 7 males 5 consumed alcohol frequently. Initial symptom in all the patients at the time of admission to hospital was dysphagia. The duration of the disease varied from one month to one year. All patients (7 males and 3 females) had squamous cell carcinoma.

The chromosomal aberrations seen in the peripheral blood leucocytes of cancer patients were chromosome gaps, chromatid gaps, acentric fragments, acrocentric associations, terminal deletion, hypodiploidy, polyploidy, double minutes, Robertsonian translocation i.e. t (D&G), loss of chromosome 2, 7q-, 10, 11, 12, 15, 17, 19, 21, Y and gain of chromosome 3, 4, 10, 19, and 22 (Table 1, Fig. 1, 2, 3 and 4). No such aberrations were seen in control samples (Table 2).

### DISCUSSION

In present study patients had low socioeconomic status and most of the males consumed alcohol. Alcohol consumption increases all indices of lipid peroxidation and may promote carcinogenesis through excessive cell proliferation induced by altered lipid and eicosonoid metabolism. Apart from genetic factors, poor nutrition, lifestyle, exposure to ultraviolet radiation or carcinogenic pollutants and alcohol abuse are suspected to be a cause of oesophageal cancer. In India low literacy rate in rural areas ensure that most patients are diagnosed at an advanced stage of the disease making it difficult to achieve cure. The results of

cytogenetic analysis showed increased numerical as well as structural abnormalities in cultured lymphocytes of oesophageal cancer patients. Most of the aberrations in leucocytes were similar to those previously reported in oesophageal tumor tissue.

Polyploidy (triploid to near tetraploid cells) was seen in 6 cases. Polyploidy as an indicator of fast growing tumor has been reported in tumor tissue of oesophageal cancer patients (Rabinovitch et al. 1989; Blount et al. 1990; Gupta 2001). Though ploidy status was associated with the advancing stage of tumor, it was not statistically associated with the differentiation of tumor (Blant et al. 2001).

Chromosome gaps were seen on chromosome 1p, 2p, and 3p in two, five and one sample respectively. Loss of chromosome number 2 was seen in one case. Interstitial deletions on 1p and 3p have been reported in tumor tissue of oesophageal squamous cell carcinoma. Loss of chromosome 2 has been also reported in tumor tissue of oesophageal cancer patients (Xiao et al. 1991). Structural and numerical aberrations of chromosome 2 have been reported in the lymphocytes of colorectal cancer (Gardner et al. 1982; Dave et al. 1993). Chromosomal loss associated with 1p, 3p, 4, 11q, 12q and gain of chromosome 12q, 17, 19 are involved in either genesis or progression of the malignancy (Pack et al. 1999).

Loss of Y chromosome was observed in two cases. Loss of Y chromosome has been also reported in tumor tissue of oesophageal cancer patients (Wang et al. 1998; Dekken et al. 1999; Mayama et al. 2000; Beuzen et al. 2000; Tang et al. 2001). The loss of Y chromosome which has a very limited genic content would make possible accumulation of 45, X, cells, making the chromosome loss a subtle indicator of any alterations of chromosome segregation. Loss of chromosome number 4 and other C group chromosomes were seen in one and two cases respectively. Loss of chromosome 7q segment in one case, chromosome 10, 11, 12, 15, 17, 19 in one case and chromosome 21 in one case were seen. Addition of chromosome 3, 10, 19 and 22 were observed in one case and chromosome 4 and 10 in one case. Numerical changes affecting chromosome 4, 6, 7, 8, 9, 10, 11, 12, 17, 18, X and Y (Persons et al. 1998) and chromosome 7, 8, 11, 17 and Y (Beuzen et al. 2000) have been reported in tumor tissue of oesophageal adenocarcinoma.

**Table 1: Profile of patients and cytogenetic results**

S. No.	Age (in years), Sex	Symptoms	Occupation	Habitat	Alcohol intake (Frequency)	Duration	Stage of Cancer analyzed	No. of cell analyzed	Abnormal cells	Type of aberration (No. of Metaphases)	Karyotype
1.	53 M	Difficulty in Swallowing food & water. Difficulty in Breathing.	Farmer	Rural	✓ Daily	3 Months	II	13	4	D&G Acrocentric association (1) Gap on 2p (2), 44 chromosomes (1)	46, XY, 44, XY,-2C
2.	49 M	Loss of weight. Vomiting in Swallowing solid food.	Farmer	Rural	X	2Months	II	19	4	D&G Acrocentric association (3) Polyploidy with 80 Chromosome (1)	46, XY
3.	95 M	Loss of weight. Difficulty in swallowing food and water. Loss of weight and appetite.	Farmer	Rural	✓ Twice in a week	1 year	IV	34	14	Acrocentric association i.e. D&G(3), G&G(1), D&2G and 3D&G(1), Double minutes (1) fragments (2), Polyploidy i.e. 75 chromosomes (1), Chromatid gap on 2p (1) 2q terminal chromatid deletion (1), Hypodiploidy (3) Gap on 1p (2) Acrocentric association i.e. D&G (2) and G&G (2)	44, XY,-4,-15,+fragments 46, XY,+10,-12 45, XY,+3,-11,-12,-17,+22 43,XY,-10,-11,-19,+fragments 46, XY
4.	65 M	Difficulty in swallowing. Loss of weight and appetite.	Farm Labour	Rural	✓ Twice in a week	5 months	II	20	6	Acrocentric association i.e. G&G(2), D&G (2) and D&D (1), Polyploidy i.e. 85 chromosomes (1)	46, XY
5.	53 M	Difficulty in Swallowing and breathing. Loss of appetite.	Textile Factory worker	Urban	X	4 months	II	30	6	Acrocentric association i.e. D&G (4), 2D&G(3), G&G(2) Fragments (3), Chromatid gap on 1p(1) and 2p(2) Loss of Y chromosome (2) Polyploidy i.e. 75 chromosomes (2) Translocation i.e. t(D;G)	46, XY 45, XY, t(D;G)
6.	65 M	Loss of weight and liquid Loss of weight and appetite.	Farmer	Rural	✓ Twice in a week	7 months	II	42	19	Acrocentric association i.e. D&G (4), 2D&G(3), G&G(2) Fragments (3), Chromatid gap on 1p(1) and 2p(2) Loss of Y chromosome (2) Polyploidy i.e. 75 chromosomes (2) Translocation i.e. t(D;G)	46, XY 45, XY, t(D;G)
7.	56 M	Difficulty in swallowing Loss of weight.	Farmer	Rural	✓ Twice in a week	6 months	II	21	12	Acrocentric association i.e. D&G (2),	46, XY 45, XY, t(D;G)

**Table 1: Contd.....**

S. No.	Age (in years), Sex	Symptoms	Occupation	Habitat	Alcohol intake (Frequency)	Duration	Stage of Cancer analyzed	No. of cell analyzed	Abnormal cells	Type of aberration (No. of Metaphases)	Karyotype
8.	45 <sup>b</sup> F	Loss of appetite. Inflammation in response to spicy food	Housewife	Rural	X	1 month	I	10	2	D&D(1), Chromosome gap on 2p(1) and Polyploidy i.e. 74 chr. (5)-Y(2) G&G Acrocentric association (1) acentric fragments (1)	46, XX
9.	32 F	Difficulty in swallowing food. Loss of weight and appetite.	Housewife	Urban	X	6 month	II	27	12	Chromosome gap on 2p (1) and 3p (1), Double minutes (2), 44 chromosomes (3), Acrocentric association i.e. D&G(3) and D&D (2)	46,XX,+19-21 44, XY,-2C
10.	55 F	Difficulty in swallowing. Loss of weight and appetite.	Housewife	Rural	X	1.5 month	I	18	10	44 chromosomes(3), Polyploidy i.e. 72 chromosomes (4), Acrocentric association i.e. D&G (3)	46,XX

<sup>a</sup>M-male, <sup>b</sup>F –female**Table 2: Profile of controls**

S. No.	Age in years	Sex	Habitat	Total No. of cells counted	No of aberrant cells	Karyotype
1	53	Male	Rural	15	None	46, XY
2	49	Male	Rural	15	None	46, XY
3	95	Male	Rural	15	None	46, XY
4	65	Male	Rural	15	None	46, XY
5	53	Male	Urban	15	None	46, XY
6	65	Male	Rural	15	None	46, XY
7	56	Male	Rural	15	None	46, XY
8	45	Female	Rural	15	None	46, XY
9	32	Female	Urban	15	None	46, XX
10	55	Female	Rural	15	None	46, XX

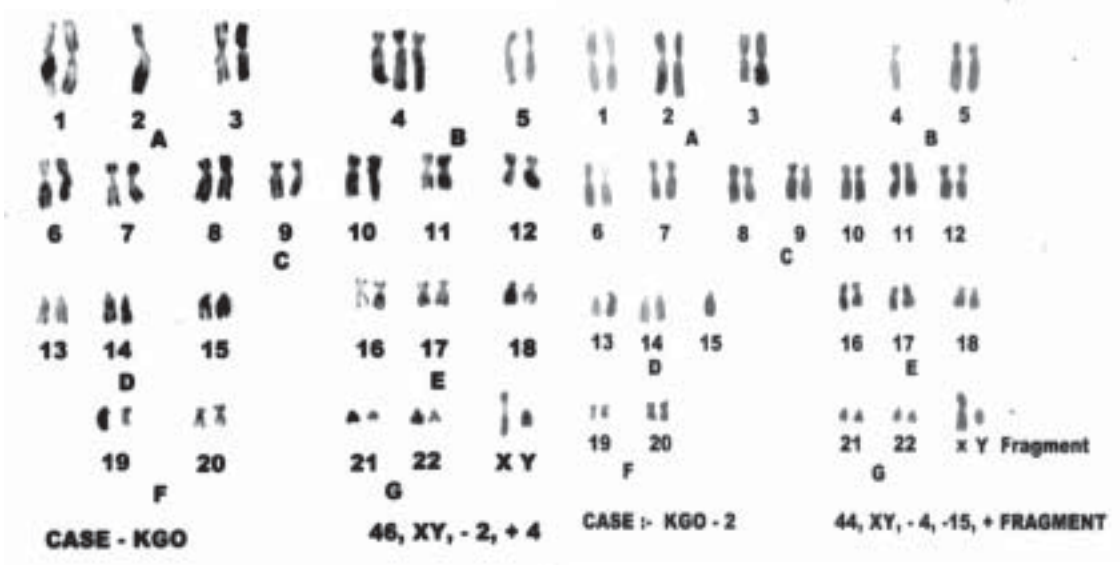


Fig. 1. CASE-KGO 46, XY, -2, +4

Fig. 3. CASE-KGO-2 44, XY, -4, -15, +Fragment

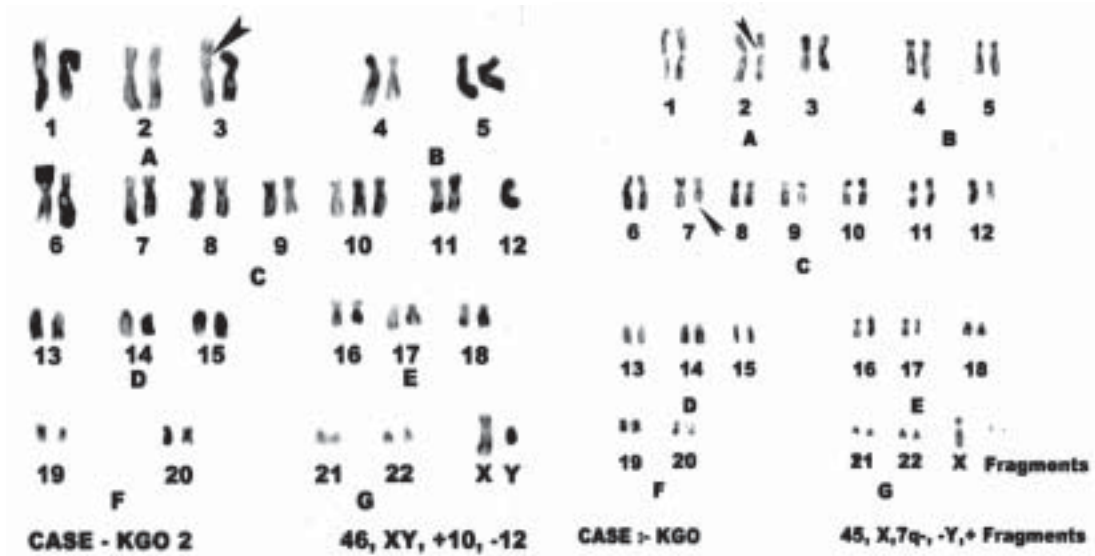


Fig. 2. CASE-KGO 2 46, XY, +10, -12

Fig. 4. CASE-KGO 45, X, 7q-, -Y, +Fragments

The authors suggested that the aberrations of chromosome 7, 8, 11, 17 and Y are associated with the early events during neoplastic transformation. Gain of chromosome 10 and loss of chromosome 15, 19 & Y have also been seen in human oesophageal cancer cell line SLMT-1 (Tang et al. 2001). Loss of heterozygosity (LOH) in 11p15.5, 11q22.3, sites of putative tumor suppressor genes have been reported in squamous cell carcinoma of oesophagus (Lam et al. 2002). LOH at 3p, 11p, 17p, 17q (Dolan et al. 1998), at 11p15 (Moskaluk et al. 1998) and 17p (Wu et al. 1998) have been reported in oesophageal adenocarcinoma. Allelic loss of 17p in oesophageal adenocarcinoma can identify patients with poor prognosis (Wu et al. 1998).

Double minutes were seen in 2 cases. These have also been reported in tumor tissue of oesophageal squamous cell carcinoma (Rosenblum-Vos et al. 1993). The cytogenetic phenomenon of double minutes has been associated with amplification of cellular oncogenes in several malignancies (Scappaticci et al. 1991). Acentric fragments were seen in 3 cases. Acentric fragments have been also reported in tumor tissue of oesophageal cancer patients (Gupta 2001). The loss or gain of chromosome or chromosome segment has been postulated to be the regions of candidate loci for tumor suppressor genes and dominantly acting growth regulatory genes.

Acrocentric associations were seen in all the cancer patients. Acrocentric associations are an indicator of tendency of acrocentric chromosomes to be involved in Robertsonian translocation. One such translocation i.e. t(D; G) was seen in one metaphase of an oesophageal cancer patient.

There are only few cytogenetic reports on leucocytes of oesophageal cancer patients. Fragile sites 1p13-36 and 4q21-q31 have been reported in cultured lymphocytes of oesophageal cancer patients. These fragile sites are believed to play an important role in oesophageal carcinogenesis. Endoreduplication and tetraploid cells have been reported in lymphocytes of oesophageal carcinoma patients (Wu 1991). High level amplification on 2p24-33, 6q21.2-q14, 7q11.2-31, 8q22-24.1, 13q2.2-34 and 8q13-qter and loss of 8p, Xp and gain on chromosome 5p have been seen in PHA stimulated lymphocytes of black and coloured population of South Africa (Du Plessis et al. 1999).

Comparative genomic hybridization (CGH) studies showed frequent gain on 1q, 2q, 3q, 7p, 11q, 12p, 12q, 17q and deletion on 1p, 3p, 4p, 11q and 17q (Yen et al. 2001), loss of 3p, 4p, 4q, 11q23-25 and gain on 1q, 3q26, 7p and 11q (Pimkhaokham et al. 2000) in squamous cell carcinoma. Loss on 3p, 4p, may be associated with early stages of tumor initiation or progression (Roth et al. 2001). It has been suggested that the chromosomal instability leads to breaks occurring at many different locations, which increase the chances of damage and could activate an oncogene to initiate a clone with chromosome rearrangement that triggers the neoplastic transformation (Brown et al. 1985). The genes implicated in oesophageal cancer and their chromosomal locations are PTGS2 (1q25.5-q25.3), ODC1 (2p25), DLEC1 (3p22-p21.3), SPARC (5q31.3-q32), EGFR (7p12), MET (7q31), FEZ1 (8p22), DMBT1 (10q25.3-q26.1), MMP7 (11q21-q22), CD9 (12p13), TOP2A (17q21-q22), cIAP1 (11q21-q23), K-ras (12p), CDKN2A (12q14) and MMP11 (22q11.2).

In conclusion, present study has revealed an increased chromosomal instability in leucocytes of oesophageal cancer patients. The aberrations are of chromosome 1p, 2p, 3p, 4, 7, 10, 11, 12, 15, 17, 19, 21 and Y. Loss or gain of chromosomes usually involved the C group chromosomes. Ten of the genes involved in oesophageal carcinogenesis are located on C group chromosomes. High frequency of aberrations involving those chromosomes that harbor these genes indicates that the oesophageal cancer patients probably have a constitutional chromosomal instability, which participates in cancer predisposition. The exact role of instability in the heterogeneity of the pathological status for a given genetic constitution can be determined by identifying the chromosomal breakpoints in tumor tissue as well as in peripheral leucocytes.

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