

Lymphocytic Chromosomal Instability in Sporadic Gastrointestinal Tract (GIT) Cancer Patients and their First-Degree Relatives

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KEYWORDS Sporadic; gastrointestinal cancer; first-degree relatives; chromosomal instability

ABSTRACT The present study was an attempt to assess utility of chromosomal instability in peripheral blood lymphocytes of first-degree relatives (FDR) of sporadic gastrointestinal tract (GIT) cancer patients for genetic surveillance. Standard lymphocyte culture technique was used for the purpose. Cultured peripheral blood lymphocyte metaphases were scored for aberrations in 10 sporadic cases of GIT cancer patients (6-esophageal, 1-gastric, 2-rectum and 1-cecum), 10 first-degree relatives and 10 healthy unrelated controls. There were significantly increased number of aberrations in cancer patients as compared to FDRs and controls. A perceptible increase in the level of metaphases with structural aberrations, including gaps, breaks, rings, centromeric separation and terminal deletions, was observed in first-degree relatives of cancer patients as compared to healthy unrelated controls taken from the same geographical area. There was high frequency of aberrations, mainly structural aberrations, involving specific chromosomes in first-degree relatives and in cancer patients. Majority of aberrations were at chromosomal loci harboring genes involved directly or indirectly in tumorigenesis, thus indicating the probability of a constitutional chromosomal instability in first-degree relatives of even sporadic GIT cancer patients.

INTRODUCTION

Instability of genome plays an important role in tumor initiation and progression. Aneuploidy is proposed as the primary cause of genome instability of neoplastic and preneoplastic cells (Langauer et al. 1997). Aneuploidy destabilizes the karyotype and thus the species, independent of mutation, because it corrupts highly conserved team of proteins that segregate, synthesize and repair chromosomes. Likewise it destabilizes genes. The transition of stable diploid to unstable aneuploid cell species is apparently the primary cause of preneoplastic and neoplastic genomic instability and of cancer, and the mutations are secondary (Duesberg et al. 2004). Chromosomal instability, leading to loss of crucial portion of DNA or loss of a complete chromosome, may participate in cancer predisposition by causing a further loss of closely linked group of genes producing proteins essential for controlled cell division. An increased risk of cancer has been seen in healthy individuals, with high levels of chromosomal aberrations in peripheral blood

lymphocytes (Boffetta et al. 2007). A significantly elevated cancer risk was observed in a Nordic cohort for subjects with both high chromosome and chromatid-type aberrations in peripheral blood lymphocytes and these variables showed equally strong predictivity (Hagmar et al. 2004). There was no significant effect of modifications by age at test, gender, country or time since test observed on the association of cancer risk with increased chromosomal instability (Bonassi et al. 2000, 2004).

A wide range of chromosomal abnormalities and microsatellite instability have been reported in tumor tissue of gastrointestinal tract (GIT) cancer patients. These include disruption of APC gene, reciprocal translocations, gain and loss of chromosomes, and rearrangements in chromosomes 1, 2, 3, 4, 5, 8, 9, 10, 11, 16, 17, 18 and 19 (Bomme et al. 1996; Popat et al. 2003; Shiomi et al. 2003; Jin et al. 2004). Studies on family members and cancer patients from high risk families using peripheral blood lymphocytes, dermal fibroblast monolayer cultures and 8 blood markers did not yield any common biomarker shared by all family members at risk of developing cancer (DiLerina et al. 1987; Guanti et al. 1990). But increased in-vitro tetraploidy was reported in skin cultures derived from patients with colon cancer syndrome and some family members at risk (Danes, 1981). Increased endoreduplication and tetraploidy was also reported in lymphocytes

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of members of high risk cancer families for esophageal carcinoma (Wu, 1991). In patients with colorectal cancers and asymptomatic first degree relatives, increased numerical and structural aberrations involving chromosome 1 and 5 were observed (Dave et al. 1993). An increased chromosomal instability and increased expression of aphidicolin and bromodeoxyuridine (BrdU) inducible fragile sites were reported in relatives of individuals affected with gastro-intestinal tumors (Tunca et al. 2000). A mutagen-induced hypersensitivity and deficient DNA repair capacity was observed in unaffected relatives of colorectal cancer patients (Ankathil et al. 1999). A bleomycin induced mutagen hypersensitivity on specific chromosomes; especially chromosome 4 was reported in first degree relatives of cancer patients (Zhu et al. 2002). An increased allelic loss from chromosome 13q was found in esophageal cancer patients with positive family history (Hu et al. 2003). These studies establish the role of genetic instability associated with family history of cancer. A high incidence of aneuploidy and presence of premature separation of centromere was observed in lymphocytes and Epstein-Barr virus (EBV) transformed lymphoblastoid cell lines from first degree relatives of breast cancer patients (Rao et al. 1996). The association between chromosomal aberration frequency and cancer risk, in particular of stomach cancer was validated by a study of 11,991 subjects of Czech, French and Italian origin (Rossner et al. 2005).

Sporadic gastrointestinal tract cancer has an increased incidence in Amritsar district (Rotary Cancer Institute, Amritsar; unpublished data). The epidemiological factors for it are unclear and the risk for first-degree relatives of cancer patients is unknown in this particular population group. There is no surveillance done yet for first-degree relatives.

The rationale of the present research is drawn from limited number of previous reports about lymphocytic chromosomal abnormalities in asymptomatic first-degree relatives of sporadic GIT cancer patients (Dave et al. 1993; Ankathil et al. 1999; Tunca et al. 2000 and Zhu et al. 2002) to assess the utility of chromosomal profile of cultured Peripheral blood lymphocytes for genetic surveillance.

MATERIAL AND METHODS

The lymphocytes of 10 sporadic cancer

patients (6-esophageal, 1-gastric, 2-rectum and 1-cecum), 10 healthy first-degree relatives and 10 healthy unrelated controls were analyzed for chromosomal aberrations. Blood samples were collected from cancer patients who had not yet undergone any treatment (surgery or chemotherapy and radiotherapy), their healthy asymptomatic relatives and controls with informed consent from Rotary Cancer Hospital, Vallah, Amritsar. The information about the subjects was collected on a pretested questionnaire. Standard lymphocyte culturing protocol (Moorehead et al. 1960) with slight modifications was used. Five ml of venous blood was drawn in heparinized syringe and kept at 4°C for 2 hours. For setting-up of lymphocyte cultures, 8 ml RPMI 1640 medium (Hi-Media), 0.15 ml phytohaemagglutinin-M (PHA-M) (Gibco), 1.5 ml autologous serum and cells from buffy coat were used. The lymphocytes were cultured for 71 hours. At 70th hour 0.025 ml of colcemid (10 µg/ml; Sigma) was added to the culture. Culture was terminated one hour after adding colcemid. Cultured lymphocytes were given hypotonic treatment with 0.075 M KCl and then fixed with 3:1 methanol:acetic acid. For each case 5-6 slides were prepared and GTG banded (Benn and Perle 1986). In total, 100 well-spread metaphases were selected per subject after analyzing different slides of the subject. Selected metaphases were scored for aberrations under microscope. Chromosomal aberrations were classified using ISCN system of nomenclature (ISCN 2005). The student's t-test was used to compare the results statistically.

RESULTS

All the subjects belonged to Amritsar district, with their residence in villages adjoining main Amritsar city. These subjects were in the age group of 22-70 years. Their economic status was lower middle class or middle class with monthly income in the range of Rs. 3,000 to Rs. 10,000. They were taking vegetarian diet and were nonsmokers and non alcoholics. The FDRs and controls were healthy and not on any medications for at least two years prior to sampling.

Gross aberrations observed in the selected metaphases were polyploidy, aneuploidy, rings, chromatid and isochromatid gaps and breaks, terminal deletions, small fragments, double minutes, centromeric separations, fragmentations

Table 1: Cytogenetic profile of cancer patients and first-degree relatives

Type of cancer, stage and duration of disease	Age and sex of cancer patients	Representative karyotype in patients	Age and sex of FDR, and relation with patient	Representative karyotype in FDRs
SCC Esophagus Stage-III 4 Months	65 M*	46, XY, +4,terdel(15q)-16/43,XY,terdel(3p), -10,-11,-12/46,XY,chtg(3p)/46,XY,chtb(1q),chtg(3q)/45,XY, isochtg(1p),chtb(5q).-21 46, XX/45,XX, -21	60 M Brother	46, XY/45,XY,terdel(3q), +11,-21,-22/46,XY, isochtg(5p)
SCC Esophagus Stage-III 3 Months	30 F**	46, XY/42,XY,-9,-11,-12,-21/44,Y,-5,-X,terdel(15q),terdel(16q)/45,XY,-17/47,XY,+14, isochtg(4q)/46,XY,chtb(1p)	70 F Mother	46, XX/46,XX,terdel(1p)/46,XX,chtb(4q)
SCC Esophagus Stage-III 1 Month	32 M	46, XY/42,XY,-9,-11,-12,-21/44,Y,-5,-X,terdel(15q),terdel(16q)/45,XY,-17/47,XY,+14, isochtg(4q)/46,XY,chtb(1p)	33 M Brother	46, XY,-3,-22/46,XY,chtg(3p)
SCC Esophagus Stage-III 2 Months	57 M	45,XY,-1,+7,+14,-21,-22/45,XY,-1,+7,-17/46,XY,+6,-21	28 F Daughter	46, XX/45,XX,-22/45,XX,-21/46,XX,chtb(2q), isochtg(3p)
SCC Esophagus Stage-III 3 Months	55 M	46, XY,terdel(2q)/42,X,-6,-11,-12,-Y/46,XY,+14,-21/45,XY,rob(22;14)/46,XY,-22,chtg(4q)/46,XY,-1,+7	35 F Daughter	46, XX/45,XX,-1,+14,rob(22;21)/46,XX, isochtg(16q)
SCC Esophagus Stage-III 2 Months	60 M	46,XY / 45,XY,-11,terdel(12p)	20 F Daughter	46, XX/46,X,terdel(2q), +22/46,XX,chtg(1p)
Gastric adeno-carcinoma Stage-I 3 Months	58 M	46,XY / 45,XY,-21,chtb(2q),chtg(12p)	20 M Son	46, XY/46,XY,del(3p),-21
Rectal polyps Stage-I 4 Months	40 F	46,XY/46,XY,terdel(6q)/45,XY,-17, isochtg(2q), isochtg(3q)	70 F Mother	46, XX/47,XX,+6,terdel(15q)/45,XX,-1,-3,chtg(6p),+20,-22/46,XY,dic(2),-3,+14/47,X,+4,+14/45,XX,terdel(1q), +6,-11,-15 46, XY/44,XY,-21,-19/46,XY,chtg(1q)
Rectosigmoid Stage-II 9 Months	48 M	46,XY/45,XY,+7,-17,-22/44,XY,-16,-18/46,XY, isochtg(1q), isochtg(2p)	22 M Son	
Cecum Stage-I 1 Month	55 M	45,XY,-21/45,XY,-1,-12,+19/46,XY,-1,+8,+15,-17,chtb(6q)	30 M	46, XY/46,XY,terdel(1p),terdel(2p)/48,XY,+3,+22/
			Son	46, XY,terdel(3p),terdel(4q),13,-20

*Male ** Female

and acrocentric associations. The first-degree relatives showed aberrations in 'A' group chromosomes including terminal deletions of 1p, 1q, 2p, 2q, 3p, or 3q, and loss of chromosome 1 and 3. A loss of chromosome 21 and 22 was also observed. In first-degree relatives, chromosome-type aberrations (numerical aberrations) were 40.17%, chromatid-type aberrations (structural aberrations) were 28.75% and acrocentric associations were 39.13% in selected metaphases (Tables 1 and 2).

The cancer patients showed chromosome-type aberrations in 41.03%, chromatid-type aberrations in 23.96% and acrocentric associations in 47.88% of selected metaphases (Table 2). Six esophageal cancer patients showed loss of chromosomes 1, 2, 5, 6, 10, 11, 12, 14, 17,

21, 22, X and Y, and terminal deletions in 2q, 3p, 12p, 15q and 16q. Gastric cancer patient showed loss of chromosome 21. Chromatid gaps and breaks were seen in 12p and 2q, respectively. Rectum cancer patients showed loss of chromosomes 17 and 22, and also terminal deletion of 6q. Cecum cancer patient showed loss of chromosome 1, 12, 17 and 21, and gain of chromosomes 8, 15, 19 (Table 1).

Gross aberrations observed in lymphocytes of healthy unrelated controls were presence of chromatid gaps in chromosome 1, breaks in chromosome 2 and centromeric separation in chromosomes 1, 2 and 3. Metaphases with numerical aberrations showed a frequency of 2.42%, structural aberrations showed 1.25% and acrocentric association showed a frequency of

Table 2: Frequency of aberrations in cancer patients and their first-degree relatives (FDRs)

Age and sex of cancer patients	Type of cancer and duration	Age and sex of FDR and relation with patient	% aberrant metaphase		% specific aberrations among the aberrant metaphases					
					Numerical		Structural		Acrocentric associations	
			Patient	FDR	Patient	FDR	Patient	FDR	Patient	FDR
65 M*	SCC Esophagus Stage-III 4 Months	60 M Brother	56	29	67.85	41.37	8.9	20.68	35.7	44.82
30 F**	SCC Esophagus Stage-III Mother 3 Months	70 F	35	15	25.7	40	34.28	6.6	54.28	33.33
32 M	SCC Esophagus Stage-III 1 Month	33 M Brother	53	20	64.1	50	18.86	15	35.84	35
57 M	SCC Esophagus Stage-III 2 Months	28 F Daughter	30	21	16.66	28.57	53.33	9.5	26.66	61.9
55 M	SCC Esophagus Stage-III 3 Months	35 F Daughter	45	28	45.44	35.71	17.77	32.14	33.33	35.74
60 M	SCC Esophagus Stage-III 2 Months	20 F Daughter	27	15	70.37	26.66	14.8	26.66	55.55	26.66
58 M	Gastric adenocarcinoma Stage-I 3 Months	20 M Son	30	10	43.33	40	10	50	63.33	40
40 F	Rectal polyps Stage-I Mother 4 Months	70 F	30	36	30	55.55	36.66	25	33.33	47.22
48 M	Rectosigmoids Stage-II 9 Months	22 M Son	35	15	22.85	26.66	8.5	73.33	86.66	46.66
55 M	Cecum Stage-I 1 Month	30 M Son	41	45	24	57.14	36.58	28.57	53.65	20
	Mean		38.2	23.4	41.03	40.17	23.96	28.75	47.88	39.13

*Male ** Female

9.31% in healthy unrelated controls (Table 3). The total number of aberrant metaphases constituted 38.20% of selected metaphases of all cancer patients, 23.40% in first-degree relatives of patients and 12.50 % in all healthy unrelated controls (Tables 2 and 3). The cancer patients had significantly higher ($t_{cal} > t_{tab}$ at $p=0.05$), aberrant metaphases than the FDRs and controls. The FDRs also had significantly higher aberrant metaphases than controls ($t_{cal} > t_{tab}$ at $p=0.05$). The frequency of structural and numerical aberrations were nearly similar in cancer patient and their first degree relatives as the difference was statistically non significant ($t_{cal} < t_{tab}$ at $p=0.05$). The acrocentric associations were significantly higher ($t_{cal} > t_{tab}$ at $p=0.05$) in cancer patients as compared to FDRs (Table 4).

DISCUSSION

In the present study, the aberrations observed in cancer patients were higher than unrelated healthy controls and difference was statistically significant. The aberrations in FDR's were also higher as compared to controls and difference was statistically significant (Table 4). An increased incidence of chromosomal aberrations involving specific chromosomes was observed in lymphocytes of cancer patients and the first-degree relatives of cancer patients. Terminal deletions, chromatid breaks and chromatid gaps involving A and B group chromosomes were common.

Chromosome loss associated with 1p, 3p, chromosome 4, 11q and 12q, and gain of chromosome 12q, 17 and 19 have been reported to be involved in either genesis or progression

of the malignancy in esophageal cancers (Pack et al. 1999). Loss of expression of P73 gene and mutations in N-ras oncogene present on chromosome 1 were associated with esophageal tumor development. In all GIT cancer patients and their first-degree relatives, there was loss of 'G' group chromosomes. In 6 cancer patients and 4 FDRs there was loss of chromosome 21. The tumor-suppressor gene TFF1 (Trefoil Factor 1) along with two other Trefoil family member genes is found in a cluster on chromosome 21. Members of the trefoil family are characterized by having at least one copy of trefoil motif, a 40-amino acid domain that contains three conserved disulphides. They are stable secretor/ check proteins expressed in gastrointestinal mucosa. Their functions are not fully defined, but they may protect the mucosa from insults, stabilize the mucus layer, and affect healing of epithelium. Loss of this gene has been previously reported in human GIT tumors (Kato 2003).

Loss of chromosomes 11 and 12 was seen in cancer patients, but such a loss was not observed in first-degree relatives, except for a single first-degree female relative (Table 1). This relative, with age 70 years, showed a high degree of spontaneous instability. Such instability was not seen in other first-degree relatives with similar age. Chromosomes 11 and 12 harbour groups of genes which are implicated in V(D)J recombination (RAG1, RAG2), tumor suppression (CDK1C, CDKN1B), malignancy of tumors (HRA, TRIM), DNA damage checkpoints (RAD9A, RAD52, CHEK1), neoplasm metastasis (LRP5), checkpoint kinases (ATM), segregation (ZW10), apoptosis (P53AIP1, APAF1, BCL2L14) and transcription factor (MDM2). Loss of whole

Table 3: Gross aberrations observed in lymphocytes of unrelated healthy controls

Age and sex of subject	Aberrant metaphases (%)	Metaphases with numerical aberrations(%)	Metaphases with structural aberrations(%)	Metaphases with acrocentric associations(%)	Representative karyotype
51 M*	11.58	3.16	1.05	7.37	46,XY,g(1),+ace
49 M	14.28	1.12	2.25	11.23	46,XY
56 M	14.28	2.38	1.19	10.71	46,XY,terdel(2)
57 M	10.23	1.14	1.14	7.95	46,XY
49 M	13.79	2.30	1.15	10.34	46,XY,terdel(3)
37 M	11.02	2.36	0.79	7.87	46,XY,+ace
42 M	16.06	2.19	2.19	11.68	46,XY
50 M	14.43	2.06	1.03	11.34	46,XY
29 M	11.43	4.28	—	7.14	46,XY,+ace
36 M	11.83	3.22	1.07	7.53	46,XY
Mean	12.50	2.42	1.25	9.31	

* Male

Table 4: Statistical comparison of aberrations in cancer patients, FDRs and controls

Categories	Mean % aberrant metaphases	Sample size	SD $\pm$ SEM	Comparable groups	t-value and significance at 5% level
Aberrant metaphases in cancer patients	38.2	10	10.21 $\pm$ 3.23	Aberrant metaphases in cancer patients and first-degree relatives	tcal > ttab significant difference
Aberrant metaphases in first-degree relatives	23.4	10	10.99 $\pm$ 3.47	Aberrant metaphases in cancer patients and healthy unrelated controls	tcal > ttab significant difference
Aberrant metaphases in healthy unrelated controls	12.5	10	1.96 $\pm$ 0.61	Aberrant metaphases in healthy unrelated controls and first-degree relatives	tcal > ttab significant difference
Numerical aberrations in cancer patients	41.03	10	10.12 $\pm$ 2.81	Numerical aberrations in cancer patients and first-degree relatives	tcal < ttab non significant difference
Numerical aberrations in first-degree relatives	40.17	10	6.66 $\pm$ 2.11	Numerical aberrations in cancer patients and healthy unrelated controls	tcal > ttab significant difference
Numerical aberrations in healthy unrelated controls	2.42	10	0.66 $\pm$ 0.18	Numerical aberrations in healthy unrelated controls and first-degree relatives	tcal > ttab significant difference
Structural aberrations in cancer patients	23.96	10	4.88 $\pm$ 1.35	Structural aberrations in cancer patients and first-degree relatives	tcal < ttab non-significant difference
Structural aberrations in first-degree relatives	28.75	10	3.56 $\pm$ 1.13	Structural aberrations in cancer patients and healthy unrelated controls	tcal > ttab significant difference
Structural aberrations in healthy unrelated controls	1.25	10	0.83 $\pm$ 0.23	Structural aberrations in healthy unrelated controls and first-degree relatives	tcal > ttab significant difference
Acrocentric associations in cancer patients	47.83	10	6.23 $\pm$ 1.73	Acrocentric associations in cancer patients and first-degree relatives	tcal > ttab significant difference
Acrocentric associations in first-degree relatives	39.13	10	4.79 $\pm$ 1.51	Acrocentric associations in cancer patients and healthy unrelated controls	tcal > ttab significant difference
Acrocentric associations in healthy unrelated controls	9.31	10	2.94 $\pm$ 0.82	Acrocentric associations in healthy unrelated controls and first-degree relatives	tcal > ttab significant difference

protein-forming machinery indicates a hyped instability with unknown origin in lymphocytes of GIT cancer patients (Mitelman 2005).

Altered chromosomes like 8p⁻, 8q⁺, 11q⁻ and +13 in esophageal carcinoma, inversion 9, del(7) and +8 in gastric cancer and chromosome arrangements characteristic of cancerous lesions have been observed previously in lymphocytes of precancerous lesions suggesting possible cancer predisposition (Jude et al. 2005). Loss of chromosomes 2, 7q, 10, 11, 12, 15, 19, 21 and Y, and gain of chromosomes 3, 4, 10, 19 and 22 have previously been reported in peripheral blood lymphocytes of esophageal cancer patients from same geographical area (Guleria and Sambyal 2003). Chromosomal aberrations involving chromosomes 2, 7, 11, 12, 15, 19, 22 and X were also reported in peripheral blood lymphocytes of GIT and breast cancers patients (Guleria et al. 2005). Structural and numerical aberrations in chromosome 1, 2, 5, 7, 12, 14, 17, 18 and 21 have been reported earlier also in lymphocytes of GIT cancer patients (Barletta et al. 1993; Dave et al. 1995; Sokova et al. 1997). It has been reported that chromosome segments frequently lost are regions of candidate loci for tumor suppressor genes and those frequently gained are candidate loci of dominantly acting growth regulator genes (Rosenblum-Vos et al. 1993).

Spontaneous chromosomal aberration in *in-vitro* cultured peripheral blood lymphocytes of pediatric cancer patients and their healthy first-degree relatives has earlier been reported which suggests an increase in chromosomal aberration in asymptomatic relatives and points towards its consideration for biomarker studies (Bakshi et al. 1999). Recurrent losses, including 1p, 14q, 10q, 13q, 15q, 18q and 22q, and gain of 5p, 12q, 17q and 20q have been reported as genetic markers with prognostic potential in gastrointestinal stromal tumors (Chen et al. 2004). Though a database is required to characterize aberrations as a marker of cancer risk (Rossener et al. 2005), there is evidence that increased frequency of chromosomal aberrations in blood lymphocytes is predictor of cancer (El-Zein et al. 2005; Boffetta et al. 2007, El-Zein, 2007; Ghosh et al. 2007).

In conclusion, the present study has revealed an increased chromosomal instability on specific chromosomes in lymphocytes of sporadic GIT cancer patients and their first-degree relatives as compared to healthy unrelated controls. In cancer patients the instability is increased probably due

to tumor progression. In first-degree relatives of GIT cancer patients, aberrations observed are similar to the aberrations reported in lymphocytes of GIT cancer patients in previous studies (Barletta et al. 1993; Dave et al. 1995; Sokova et al. 1997; Pack et al. 1999; Guleria and Sambyal 2003; Chen et al. 2004; Guleria et al. 2005; Jude et al. 2005). A high frequency of aberrations involving specific chromosomes harboring particular genes involved, directly or indirectly, in tumorigenesis, indicates a constitutional chromosomal instability. Thus first-degree relatives of sporadic cancer patients are fundamentally a risk group and cytogenetic analysis of lymphocytes as an adjunct to other tests has the potential to be used for surveillance of these subjects.

ACKNOWLEDGEMENT

UGC grant F.31-267/2005 is gratefully acknowledged.

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