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Original Article

Increased Sister Chromatid Exchanges in Patients with Gastrointestinal Cancers and in their First-Degree Relatives

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ABSTRACT

Gastrointestinal Cancers (GICs) are the most important causes of mortality and morbidity in industrialized world. Sister chromatid exchange (SCE), as an index of chromosomal instability, involves cancer. The aim of this study is to determine whether SCE frequency is a heritable factor for GIC or not. The study groups consisted of 15 gastrointestinal carcinoma patients, 13 patient relatives and 15 healthy subjects as the control group. After collection of 2 ml peripheral blood, lymphocytes were cultured for 3 days and sister chromatid exchange (SCE), mitotic index, and replication index were analyzed. SCE was significantly increased (p<0.01) in patients (16.06±22.37) and in their relatives (5.23±2.64) compared with controls (3.51±1.58). There was no significant difference between patients' relatives and control group in terms of the incidence of SCE frequency. Mitotic index was significantly decreased (p<0.05, p<0.01) in patients (5.4±3.13) compared with healthy relatives (7.15±2.15) and controls (9.00±2.26). Replication index was also significantly lower (p<0.01) in patients (1.39±0.35) and in their relatives (1.7±0.21) compared with controls (2.04±1.13). The results of this study indicate that SCE is a heritable factor for GICs. Increased SCE reflects genomic instability, which is an important factor in carcinogenesis. Although the most putative factors causing genomic instability are epigenetics marks, further studies in combination with epigenetic modifications are needed using more subjects.

Key words: *Sister chromatid exchange, chromosomal instability, gastrointestinal cancer*

Birinci Derecede Akrabalarda Gastrointestinal Kanserli Hastalarda Artmış Kromatid Değişimler

ÖZET

Gastrointestinal kanserler, sanayileşmiş ülkelerdeki mortalite ve morbiditenin en önemli sebeplerinden biridir. Kromozomal kararsızlığın ölçüsü olan kardeş kromatid değişimi (KKD) kanser etiyolojisinde yer almaktadır. Bu çalışmanın amacı, kardeş kromatid değişim sıklığının gastrointestinal kanserde kalıtsal bir faktör olup olmadığının araştırılmasıdır. Çalışma grupları 15 gastrointestinal kanser hastası ve 13 hasta yakını, kontrol grubu ise 15 sağlıklı bireyden oluşmaktadır. Olgulardan 2 ml periferik kan alındıktan sonra lenfositler 3 gün süreyle kültüre edildikten sonra, kardeş kromatid değişimi, mitotik indeks ve replikasyon indeksi analiz edildi. Kardeş kromatid değişimi açısından hasta (16,06±22,37) ve hasta yakınları (5,23±2,64) grubunda kontrol grubuna (3,51±1,58) göre istatistiksel olarak anlamlı bir artış gözlemdi (p<0,01). Hasta grubunun (5,4±3,13) mitotik indeksi hasta yakınları (7,15±2,15) ve kontrol grubuna (9,00±2,26) göre anlamlı derecede düşük çıkmıştır (p<0,05, p<0,01). Benzer olarak hasta grubunun replikasyon indeksi (1,39±0,35), hasta yakınları (1,7±0,21) ve kontrol grubuna (2,04±1,13) göre anlamlı derecede düşük çıkmıştır (p<0,01). Elde edilen sonuçlar kardeş kromatid değişiminin gastrointestinal kanserler için kalıtsal bir faktör olduğunu göstermektedir. Artan kardeş kromatid değişimi, karsinogenezde etken olduğu bilinen genomik kararsızlığın ölçüsüdür. Fakat genomik kararsızlığın başlıca sebepleri arasında epigenetik değişiklikler olduğundan, epigenetik değişikliklerle kombine daha ileri araştırmaların yapılması gerekmektedir.

Anahtar kelimeler: *Kardeş kromatid değişimi, kromozomal kararsızlık, gastrointestinal kanser*

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INTRODUCTION

GICs were the fourth most common cancer (accounting for 988.602 cases) in 2008, and they were estimated to reach 1.1 million in 2010. GICs are the second leading causes of cancer-related death worldwide due to late detection and high recurrence rates. Today, these cancers have a heavy socioeconomic burden, and the pathophysiological features of cancer should be understood in detail for promising biomarkers and therapeutic targets. The development of gastric cancer in people has been shown to be a multi-step process, ranging from chronic gastritis to atrophy, intestinal metaplasia, dysplasia and finally, invasive cancer (1, 2, 3). The biggest challenge to overcome cancer is the tumor cells heterogeneity. The underlying reasons of this heterogeneity should be clarified for risk analysis. One of the main putative reasons for heterogeneity is instability in sister chromatid. According to semi-conservative replication theory, the DNA sequence of sister chromatids should be identical except for errors. However, the epigenetic marks of sister chromatids are not identical, and they are randomly distributed between sister chromatids. These differences may result from epigenetic variations in progenitor cells (4, 5).

The chromosomal instability of cancer was analyzed through many methods including cytogenetic, molecular cytogenetic and molecular genetic methods. Of these methods, the sister chromatid exchange (SCE) is the most appropriate method for conducting an analysis. SCE is defined as the reciprocal exchange of segments between sister chromatids. The measurement of SCE in peripheral blood lymphocytes was for many years one of the most popular cytogenetic methods for evaluating human genotoxicity and hereditary disease (6, 7, 8). It has been reported that Bloom's Syndrome lymphocytes, oral submucous fibrosis, ovarian cancer, malignant mesothelioma, cervical cancers, malignant melanoma, and breast cancer were analyzed through manipulated SCE incidence (9-14). The aim of this study is to determine whether SCE frequency, as an index of chromosomal instability, is a heritable factor for GIC or not. To this end, the lymphocytes obtained from GIC patients, their relatives and control groups were cultured and analyzed. Mitotic index and replication index were also calculated.

MATERIALS AND METHODS

This study was conducted at Düzce University Medical Faculty, Department of Medical Genetics, Düzce, Turkey. The study groups consisted of 15 GIC patients (2 esophagus, 6 stomach, 6 colon and 1 rectum), 13 relatives and 15 healthy subjects. Approved ethical certificate was obtained from local ethics committee, and written informed consents of the studied cases were obtained. Age, gender, smoking and alcohol consumption status and tumor markers (alpha-fetoprotein, AFP, carcinoembryonic antigen-CEA, carcinogenic antigen (CA) 125, CA19-9, CA15-3) were recorded. Two milliliters of venous blood was drawn using Na-heparinized syringes from each case. Peripheral blood lymphocytes were incubated in the culture medium Chang Medium MF (Irvine Scientific) + Phytohaemagglutinin M (PHA-M) (Biological Industries) for 72 hours. Bromodeoxyuridine (BrdU; Sigma Chemical Company, USA) was added to each flask at the 24th hour of culture, and then lymphocytes incubated for another 48 hours in darkness. At the end of the 72 hours, cell was harvested with standard fluorescence plus Giemsa techniques, and then metaphase figures were obtained (8, 15). For each patient, 20 metaphase spreads were assessed with a light microscope (100X). Mean SCE frequency per metaphase was calculated for patients, their first-degree relatives and healthy control. The replication stages of chromatid were evaluated for BrdU staining. BrdU taken chromatid seems in light color under microscope. Both chromatid exchanged (light color) counted as R3, one chromatid exchanged counted as R2, no chromatid exchanged counted as R1 (dark color).

Replication Index was calculated by following formula.

RPI: R1 + (R2x2) + (R3x3) / R1 + R2 + R3

Mitotic index (MTI) was calculated by following formula.

MTI: metaphase/metaphase+lymphocyte count

Statistical analysis

The frequency of SCE per metaphase in groups was compared by Kruskal Wallis. Mann-Whitney U test was also used. Relationships between parameters were evaluated with the Spearman's correlation analysis. Qualitative data were compared using Chi-square test. Results with 95% confidence interval and $p<0.05$ were considered significant.

Table 1. Demographic features of groups

	Patients n (%)	Relatives n (%)	Control n (%)	Total n (%)
Age (years)	62.33±15.09	37.85±8.99	59.20±9.52	53.84±15.63
Male	8 (53.3)	7 (53.8)	8 (53.3)	23 (53.5)
Female	7 (46.7)	6 (46.2)	7 (46.7)	20 (46.5)
Smoke	6 (40.0)	6 (50.0)	5 (33.3)	17 (40.5)
Total	15	13	15	43

RESULTS

This study was conducted on 43 cases in total, including 23 (% 53.5) males and 20 (% 46.5) females. The demographic features of the patients, their first-degree relatives and healthy control group are shown in Table 1. The ages of patients and their relatives were significantly lower than those of the control group (p=0.001, p<0.01). There was no difference between patient's first-degree relatives and control groups in mean SCE frequency (5.23±2.64 and 3.51±1.58 per metaphase, respectively; p=0.062); however the mean frequency of patients (16.06 ± 22.37) was significantly higher than that of first-degree relatives and controls (p=0.024 and p= 0.001 respectively) (Table 2, Figure 1).

The mean MTI level of the patients was significantly lower than that of first-degree relatives and controls (5.40 ± 3.13, 7.15 ± 2.15, and 9.00 ± 2.26 respectively; p=0.044; p<0.05 and P=0.002; p<0.01 respectively). In contrast, there was no difference between first-degree relatives and controls in mean MTI levels (7.15 ± 2.15 and 9.00 ± 2.26 respectively; p=0.053; p> 0.05) (Table 2, Figure 1).

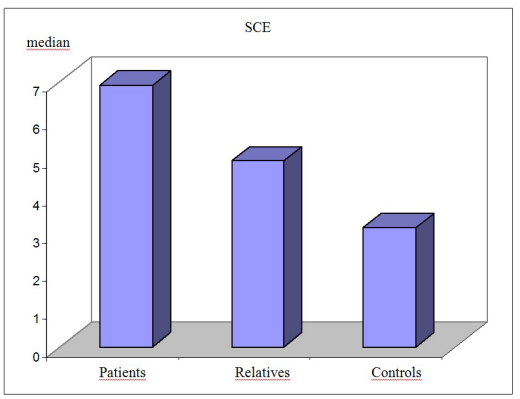

***Figure 1**. SCE distribution of measurements according to groups*

Similarly, the mean RPI level of the patient group was significantly lower than that of first-degree relatives and controls (1.39 ± 0.35, 1.70 ± 0.21, and 2.04 ± 1.13 respectively; p=0.003; p <0.01 and p=0.001; p<0.01 respectively). In contrast, there was no difference between first-degree relatives and control groups in mean RPI levels (7.15 ± 2.15 and 9.00 ± 2.26 respectively; p= 0.596; p>0.05) (Table 2, Figure 1).

6 (% 40.0) cases of 15 patients, 6 (% 50.0) cases of 13 first degree relatives and 5 (% 33.3) cases of 15 healthy subjects in control group are smokers (Table 1). The mean SCE frequency was not significant between smokers and non-smokers (data not shown).

DISCUSSION

Cancer is a multifactorial disease which affects patients' lives in different ways (16). The gastrointestinal (esophageal, gastric, pancreatic, hepatic and colorectal) cancers are among the most frequently diagnosed cancers and they cause most deaths in industrialized world (17). Among GICs, colorectal cancer is the leading cause of cancer mortality, but early diagnosis may reduce mortality by 15% - 33% (18-20). Although the rates of incidence and mortality have fallen dramatically over the last 50 years, stomach cancer is still the second most common cause of death from cancer worldwide. Esophageal cancer has also been reported at different rates in different regions of the world (19, 21). Esophageal cancer is the most incurable disease at the time of diagnosis. Among the reasons for this are late diagnosis, quick spread along the esophagus and late admission to physician. 5-year survival rates following surgical treatment are reported to be between 12 and 22% (22, 23). As a result of screening in the early stages, 5 years survival rates for this disease increase (24).

The importance of early diagnosis in cancer has been appreciated much better due to the lack of the appropriate therapy for cancer. Therefore, many studies relating

Table 2. *Mean and Standard Deviation Values of Erythrocytic Parameters of the Groups*

	Patients n (%)	Relatives n (%)	Control n (%)	P+
SCE	16.06 ± 22.37	5.23 ± 2.64	3.51 ± 1.58	0.001**
MTI	5.40 ± 3.13	7.15 ± 2.15	9.00 ± 2.26	0.002**
RPI	1.39 ± 0.35	1.70 ± 0.21	2.04 ± 1.13	0.001**

+Kruskal Wallis Test ***p<0.01 advanced significant*

to putative heritable factors in pathogenesis of the cancer have been conducted. The heritable chromosomal instability has become one of the putative targets. Up to date, the association between SCE frequency as an index of genomic instability and several cancer types has been reported. Increased SCE frequency for people carrying high risk of cancer may result from mutagens or heritable manner. To test whether the SCE frequency is a heritable factor for cancer pathogenesis, the SCE frequencies of patients with GIC, their first degree relatives and the control group were compared. It was found that the SCE levels of patient group were significantly higher than those of the relative group ($p=0.024$, $p<0.05$) and control group ($p=0.001$, $p<0.01$). However, there was no significant difference between the relatives of the patients and control groups ($p=0.062$, $p>0.05$). The mitotic index and replication index have increased tendency due to damaged DNA in cancer patients. Hence, the mitotic index and replication index are proportional to the SCE. Similar results were found in breast cancer patients by Cefle et al. They found that the SCE level of control group significantly lowers than patients and their relatives. However, the frequencies of SCE were not different between patients and their relatives (15). In contrast to our study, they found a significant difference between the relative group and the control group. The average age of the relative group was higher than the control group in this study. It may be the reason why there was no significant difference between the relative group and the control group in this study.

Karaman et al reported significantly elevated SCE frequencies in both H pylori-negative gastric cancer and H pylori-negative chronic atrophic gastritis patients compared with controls (2). These findings are consistent with our study. The chromosomal instability differences between sisters chromatids may result from epigenetics modification on it. The field of epigenetics investigates the modifications causing changes in gene expression or cellular phenotype in heritable manner without changes in DNA nucleotide sequence. The most well-known epigenetic modifications are DNA methylations, histone modification and micro RNA. These epigenetic modifications are involved in vital biological processes. Several investigations showed that epigenetic changes are taken into consideration in cancer. Therefore, the researchers focused on the putative effects of the epigenetic changes in cancer for development of early diagnosis methods and new therapeutic approaches (25, 26). This new perspective was proven by Vera et al. They found an association between DNA methylation of global/subtelomeric region and telomere-SCE frequency (27). In conclusion, SCE in venous blood could be used as a predictive test in patients with gastrointestinal cancers. However, the main limitation of this study is the number of participants. This study population comprised limited number of subjects and further investigations in combination with epigenetic modifications are needed with more subjects.

REFERENCES

1. *Li L, Ying XJ, Sun TT, et al. Overview of Methodological Quality of Systematic Reviews about Gastric Cancer Risk and Protective Factors. Asian Pacific J Cancer Prev 2012; 13:2069-79.*
2. *Karaman A, Binici DN, Kabalar ME, Dursun H, Kurt A. Alteration of sister chromatid exchange frequencies in gastric cancer and chronic atrophic gastritis patients with and without H pyroli infection. World J Gastroenterol 2008; 14(16):2534-9.*
3. *Cao DX, Li ZJ, Jiang XO, et al. Osteopontin as potential biomarker and therapeutic target in gastric and liver cancers. World J Gastroenterol 2012; 18(30):3923-30.*
4. *Feinberg AP. Phenotypic plasticity and the epigenetics of human disease. Nature 2007; 447(7143): 433-40.*
5. *Lansdorp PM, Falconer E, Tao J, Brind'Amour J, Naumann U. Epigenetic differences between sisters chromatids? Ann N Y Acad Sci 2012;1266:1-6.*
6. *Bonassi S, Lando C, Ceppi M, Landi S, Rossi AM, Barale R. No association between increased levels of high-frequency sister chromatid exchange cells (HFCs) and the risk of cancer in healthy individuals. Environ Mol Mutagen 2004; 43(2):134-6.*

7. Pitarque M, Carbonell E, Lapeña N, et al. SCE analysis in peripheral blood lymphocytes of a group of filling station attendants. Mutat Res 1997; 390(1-2):153-9.

8. Oztürk S, Palanduz S, Cefle K, et al. Genotoxicity and sister chromatid exchange in patients with myelodsplastic disorders. Cancer Genet Cytogenet 2005; 159(2):148-50.

9. Ghosh PK, Madhavi R, Guntur M, Ghosh R. Sister Chromatid Exchanges in Patients with Oral Submocous Fibrosis. Cancer Genet Cytogenet 1990; 44(2):197-201.

10. Baltaci V, Kayikcioglu F, Alpas I, Zeyneloglu H, Haberal A. Sister chromatid exchange rate and alkaline comet assay scores in patients with ovarian cancer. Gynecol Oncol 2002; 84(1):62-6.

11. Atalay F, Baltaci V, Alpas I, Savas I, Atikcan S, Balci S. Sister chromatid exchange rate from pleural fluid cells in patients with malignant mesothelioma. Mutat Res 2000; 465(1-2):159-63.

12. Dhillon VS, Kler RS, Dhillon IK. Choromosome instability and sister chromatid Exchange SCE studies in patients with carcinoma of cervix uteri. Cancer Genet Cytogenet 1996; 86(1):54-7.

13. Illeni MT, Rovini D, Grassi C, et al. Sister Chromatid Exchange analysis in familial groups of malignant melanoma patients. Cancer Genet Cytogenet 1991; 53(2):237-46.

14. Dhillon VS, Bhasker R, Kler RS, Husain SA. Sister Chromatid Exchange SCE studies in breast cancer patients; a follow-up study. Cancer Genet Cytogenet 1995; 80(2):115-7.

15. Cefle K, Ucur A, Guney N, et al. Increased sister chromatid exchange frequency in young women with breast cancer and in their first-degree relatives. Cancer Genet Cytogenet 2006; 171(1):65-7.

16. Karabulutlu EY, Bilici M, Cayir K, Tekin SB, Kantarci R. Coping, Anxiety and Depression in Turkish Patients with cancer. European Journal of General Medicine 2010; 7(3):296-302.

17. Blanco-Calvo M, Calvo L, Figueroa A, Haz-Conde M, Antón-Aparicio L, Valladares-Ayerbes M. Circulating MicroRNAs: Molecular Microsensors in Gastrointestinal Cancer. Sensors (Basel) 2012; 12(7):9349-62.

18. Bolocan A, Ion D, Ciocan DN, Paduraru DN. Prognostic and predictive factors in colorectal cancer. Chirurgia (Bucur) 2012; 107(5):555-63.

19. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61(2):69-90.

20. Yaşar M, Kayıkçı A. Retrospective Evaluation of Colonoscopy Results. Konuralp Med J 2010; 2(3):6-9.

21. Nagini S. Carcinoma of the stomach: A review of epidemiology, pathogenesis, molecular genetics and chemoprevention. World J Gastrointest Oncol 2012; 4(7):156-69.

22. Earlam R,Cunha-Melo JR. Oesophageal squamous cell carcinoma I: a critical review of surgery. Br J Surg 1980; 67(6):381-90.

23. King RM, Pairolero PC, Trastek VF, Payne WS, Bernatz PE. Ivor Lewis esophagogastrectomy for carcinoma of the esophagus: early and late functional results. Ann Thorac Surg 1987; 44(2):119-22.

24. Isono K, Sato H, Nakayama K. Results of nationwide study on the three field lymph node dissection of esophageal cancer. Oncology 1991; 48(5):411-20.

25. Feinberg AP, Ohlsson R, Henikoff S. The epigenetic progenitor origin of human cancer. Nat Rev Genet 2006; 7(1):21-33.

26. Hatziapostolou M, Iliopoulos D. Epigenetic aberrations during oncogenesis. Cell Mol Life Sci 2011; 68(10):1681-702.

27. Vera E, Canela A, Fraga MF, Esteller M, Blasco MA. Epigenetic regulation of telomeres in human cancer Oncogene 2008; 27(54):6817-33.