ACTA
STOMATOLOGICA
CROATICA
www.ascro.hr

Acta Stomatol Croat. 2008;42(4):318-325.

IZVORNI ZNANSTVENI RAD
ORIGINAL SCIENTIFIC PAPER

Sertan Ergun[1], Hakkı Tanyeri[1], Şükrü Öztürk[2], Nilgün Duman[3], Sevil Kamal[4], Ahmet Gül[4], Reyhan Küçükkaya[5], Sevda Özel[6], Kıvanç Çefle[2], Şükrü Palanduz[2]

Ispitivanje genetske nestabilnosti primjenom analize razmjene sestrinskih kromatida kod pacijenata sa Sjögrenovim sindromom

Investigation of Genomic Instability in Patients with Sjögren's Syndrome by Using Sister Chromatid Exchange Analysis

[1] Zavod za oralnu medicinu i kirurgiju Stomatološkog fakulteta Sveučilišta u Istambulu
Istanbul University, Faculty of Dentistry, Department of Oral Medicine and Surgery
[2] Zavod za internu medicinu - Odjel za medicinsku genetiku Medicinskog fakulteta Sveučilišta u Istambulu
Istanbul University, Istanbul Faculty of Medicine, Department of Internal Medicine, Division of Medical Genetics
[3] Institut za zdravstvenu znanost - Zavod za genetiku Sveučilišta u Istambulu
Istanbul University, Institute of Health Science, Department of Genetics
[4] Zavod za internu medicinu - Odjel za reumatologiju Medicinskog fakulteta Sveučilišta u Istambulu
Istanbul University, Istanbul Faculty of Medicine, Department of Internal Medicine, Division of Rheumatology
[5] Zavod za internu medicinu - Odjel za hematologiju Medicinskog fakulteta Istambulskog sveučilišta
Istanbul University, Istanbul Faculty of Medicine, Department of Internal Medicine, Division of Hematology
[6] Zavod za javno zdravstvo - Medicinski fakultet Istambulskog sveučilišta
Istanbul University, Istanbul Faculty of Medicine, Department of Public Health

Sažetak

Svrha rada bila je ispitati genetsku nestabilnost limfocita iz perifernog krvotoka primjenom razmjene sestrinskih kromatida (SCE-a) kod pacijenata sa Sjögrenovim sindromom (SS-om). **Pacijenti i metode**: ispitano je koliko je čest SCE (po metafazi) kod pacijenata s primarnim Sjögrenovim sindromom (n=30), zatim kod neliječenih pacijenata (n=15) i među zdravim pojedincima (n=15) u kontrolnim skupinama. **Rezultati:** vrijednosti srednje učestalosti SCE-a koje smo dobili za pacijente sa SS-om, kod onih s neliječenim limfomom i kod zdravih u kontrolnoj skupini, iznosile su 7,77±1,50, 8,80±0,75 i 6,65±1,50. Dobili smo statistički znatne razlike između pacijenata sa SS-om i onih s limfomom (p=0,007) te između pacijenata s limfomom i zdravih sudionika iz kontrolnih skupina (p=0,0001). Nisu zapažene statistički velike razlike između pacijenata sa SS-om i onih u kontrolnim skupinama, s obzirom na srednju čestoću SCE-a (po metafazi). **Zaključak:** poznato je da se kod oko pet posto svih pacijenata sa SS-om mogu razviti limfomi. Česti SCE smatra se citogenetskim biomarkerom ranoga mutagenog učinka koji upućuje na povećani rizik od raka. Uočili smo da su vrijednosti srednje učestalosti razmjene sestrinskih kromatida (po metafazi) kod triju pacijenata sa SS-om bile više u odnosu prema vrijednostima ustavnovljenima kod pacijenata s neliječenim limfomom. Rezultat se može provjeriti procjenom drugih parametara s važnom zadaćom u malignoj transformaciji.

Zaprimljen: 14. veljače 2008.
Prihvaćen: 12. listopada 2008.

Adresa za dopisivanje
Sertan Ergun, D.D.S, Ph.D.
Istanbul University
Faculty of Dentistry
Department of Oral Medicine and Surgery
34393 Capa, Istanbul
Turkey
sertanergun@gmail.com

Ključne riječi
Sjögrenov sindrom; sestrinske kromatide, razmjena; limfom

Uvod

Sjögrenov sindrom (SS) kronična je i organski specifična autoimuna bolest kojoj je svojstvena limfocitna infiltracija žlijezda slinovnica i suznih žlijezda (1-3). Manifestacije su keratoconjunctivitis sicca (suhe oči), xerostomia (suha usta) i druge izvanžlijezdane abnormalnosti (4-6). Definiciju primarnog SS-a predstavljaju sve te pojave bez dodatnih bolesti vezivog tkiva (7). Sekundarni SS vezan je za reumatoidni artritis (RA), sistemski lupus eritematosus (SLE) ili druge bolesti vezivnog tkiva (7).

SS možemo podijeliti u tri stadija prema proširenosti organskih oštećenja i tijeku bolesti. U prvom stadiju (oko 45 posto slučajeva) pacijenti imaju samo sindrom sicca i nemaju znakove sustavnih oštećenja. U drugom stadiju (oko 50 posto slučajeva) javlja se limfocitno organsko oštećenje. Konačno, u trećem stadiju (oko 5 posto slučajeva) kod bolesnika se razvijaju maligni limfomi (8,9). Za limfome se smatra da imaju ishodište u limfoepitelnim lezijama u kojima se događa bliska interakcija između epitelnih stanica, stanica T i stanica B.

Pojava SS-A/Ro-a, SS-B/La-a autoprotutijela i antinuklearnih antitijela (ANA-a) u SS-u vezana je za rani početak bolesti, njezin dulji tijek, češće izvanžlijezdane manifestacije, intenzivniju limfocitnu infiltraciju žlijezda slinovnica i već navedenu predispoziciju progresiji (10, 11).

Analiza razmjene sestrinskih kromatida (SCE) sofisticirana je citomolekularna tehnika i primjenjuje se u studijama o procjeni genotoksičnosti i genetske nestabilnosti. Velika učestalost SCE-a smatra se biomarkerom ranoga mutagenog učinka, što upućuje na povećani rizik od raka (12).

Ispitanici i metode

Primjenom analize SCE-a procijenili smo genomsku nestabilnost kod pacijenata sa SS-om bez simptoma limfoma (n=30), onih s neliječenim limfomom (n=15) i zdravih pojedinaca (n=15). U skupini pacijenata sa SS-om (1) 22 pacijenta bila su upućena k nama na labijalnu biopsiju s Odjela za reumatologiju Zavoda za internu medicinu i iz Zavoda za oftalmologiju Medicinskog fakulteta Istambulskog sveučilišta s preliminarnom dijagnozom SS-a; (2) došlo je i dvadeset pacijenata s dijagnozom SS-a prema kriterijima Studijske skupine Europske unije (European Community Study Groupe - ECSG-a) koja djeluje na Odjelu za reumatologiju (13) Zavoda za internu medicinu. Od ukupno 42

Introduction

Sjögren's syndrome (SS) is a chronic organ specific autoimmune disease characterized by lymphocytic infiltration of the salivary and lachrymal glands (1-3). SS is manifested by keratoconjunctivitis sicca (dry eye), xerostomia (dry mouth) and other extraglandular abnormalities (4-6). Primary SS is defined as the presence of the above manifestations without additional connective tissue disease (7). Secondary SS is associated with rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) or other connective tissue diseases (7).

SS can be divided into three stages according to the extent of organ damage and the course of the disease. In stage I (approx. 45.0% of cases), patients have only sicca syndrome and do not experience any systemic involvement. In stage II (approx. 50.0% of cases), patients are affected by lymphocytic organ damage. Finally, in stage III (approx. 5.0% of cases) patients develop malignant lymphomas (8, 9). Lymphomas are thought to arise from lymphoepithelial lesions in which there are close interactions between epithelial cells, T cells and B cells.

The presence of SS-A/Ro, SS-B/La autoantibodies and antinuclear antibodies (ANA) in SS is associated with early disease onset, longer disease duration, higher frequency of extraglandular manifestation, more intensive lymphocytic infiltration of salivary glands and predisposition to progression described above (10, 11).

Sister Chromatid Exchange (SCE) analysis is a sophisticated cytomolecular technique and has applications in studies in which genotoxicity and genetic instability are evaluated. High frequency of SCE is regarded as a biomarker of early mutagenic effect suggesting increased risk of cancer (12).

Patients and Methods

We evaluated genomic instability in SS patients with no symptoms of lymphoma (n=30) and untreated lymphoma patients (n=15) and healthy individuals (n=15) by using SCE analysis. Patients with SS consisted of (1) 22 patients who were referred from Istanbul University, Faculty of Medicine, Department of Internal Medicine, Division of Rheumatology and Department of Ophthalmology to our department with a preliminary diagnosis of SS for taking labial biopsy, (2) twenty patients were diagnosed with SS according to European Community Study Group (ECSG) for SS criteria by Department of Internal Medicine, Division of Rheumatology (13). Out of these 42 patients with SS we chose

pacijenta sa SS-om izabrali smo 30 i to prema inkluzijskim kriterijima naše skupine ispitanika prema sljedećem opisu:

- dijagnoza SS-a postavljena prema ECSG-u
- nepušači bez povijesti pušenja
- bez malignosti u povijesti bolesti
- bez izloženosti citotoksičnim kemikalijama ili drogama s učinkom na učestalost SCE-a
- bez infekcije tijekom ispitivanja.

Prva i druga kontrolna skupina imale su po 15 pacijenata s limfomom (3 s Hodgkinovim i 12 s non-Hodgkinovim limfomom) i 15 zdravih žena.

Analiza razmjene sestrinskih kromatida (SCE-a) obavljena je u citogenetskom laboratoriju Odjela za medicinsku genetiku Zavoda za internu medicinu Medicinskog fakulteta Sveučilišta u Istambulu. Koristila se tehnika mikrokulture koju su razvili Moorhead i suradnici (14). Količina od 0,5 ml heparizinirane krvi dobivene od pacijenata i sudionika u kontrolnoj skupini dodana je mediju kulture koja sadržava RPMI 1640 (Biochrom KG, Berlin, Njemačka) pojačan s 10-postotnim fetalnim bovinim serumom i 1-postotnim PHA-om (Njemačka P-8139), a dodan je bio i 1-postotni Gentamycin. Nakon 24 sata mediju je dodano 0,5 μg/ml 5'-bromodeoksiuridina (BrdU, Sigma Chemical Company, SAD) te je daljna inkubacija obavljena u mraku. Nakon 70 sati dodan je i kolkicin (0,2μ/ml) (Colchicine u prahu, Sigma Chemical Co, SAD). Zatim su poslije 72 sata prikupljene i tretirane 10 minuta otopinom 0,75 M KCl-a na temperaturi od 37°C te fiksirane metanolnom octenom kiselinom (Merck, Darmstadt, Njemačka). Nakon toga je obavljen standardni postupak prikupljanja. Preparati su obojeni tehnikom Florescein plus Giemsa (FPG). Terminalne promjene računali smo kao jednu razmjenu, a intersticijske kao dvije. Za svakog pacijenta i svakoga iz kontrolne skupine analizirano je 30 metafaza, te je izračunato i koliko je česta razmjena sestrinskih kromatida po metafazi.

Od statističkih metoda primijenili smo studentov t-test (t) i U-testove prema Mann-Whitneyu (z) za usporedbu dviju skupina, a kod usporedbe triju skupina koristili smo se jednosmjernom analizom varijance (ANOVA) i Kruskal-Wallisovom analizom varijance (χ^2). Kvalitativni parametri uspoređeni su Kruskal-Wallisovom analizom varijance (χ^2) i Fisherovim testom (p).

Rezultati

Procijenjeno je sedam pacijenata s primarnim SS-om prosječne dobi 41,14±16,99 godina; 23 pacijenta sa sekundarnim SS-om prosječne dobi 52,7±12,1 godina; 3 pacijenta s Hodgkinovim limfomom (20 %);

30 patients with SS according to the inclusion criteria of our study group described below:

- SS diagnosed according to ECSG
- No history of smoking
- No history of malignancy
- No exposure to cytotoxic chemicals or drugs which are known to affect the frequency of SCE
- No infection during study time

Our first and second control groups consisted of 15 patients with lymphoma (3 patients with Hodgkin's lymphoma, 12 patients with non-Hodgkin's lymphoma) and 15 female healthy individuals, respectively.

Sister Chromatid Exchange (SCE) analysis was performed in the cytogenetic laboratory of Istanbul University, Faculty of Medicine, Department of Internal Medicine, Division of Medical Genetics. The micro-culture technique developed by Moorhead et al. was used (14). 0.5 ml heparinized blood obtained from patients and controls was added to the culture medium containing RPMI 1640 (Biochrom KG, Berlin, Germany), supplemented with 10% Fetal Calf Serum, 1% PHA (Germany P-8139) and 1% Gentamycin was added to the culture medium. At the 24 hours 0.5 μg/ml 5'-bromodeoxyuridine (BrdU, Sigma Chemical Company, USA) was added to the medium and it was further incubated in the dark. Colchicine (0.2μ/ml) (Colchicine powder, Sigma Chemical Co, USA) was added at the 70th hour. Then, cells were collected at the 72nd hour and treated with 0.075 M KCl at 37°C for 10 minutes, then fixed with methanol-acetic acid (Merck, Darmstadt, Germany) and standard harvest procedure was performed. Slides were stained by Florescein plus Giemsa technique (FPG). Terminal changes were counted as one, interstitial changes as two exchanges. 30 metaphases were analyzed for every patient and control cases. The frequencies of sister chromatid exchanges per metaphase were computed.

We used student–t (t) and Mann-Whitney U tests (z) for comparing two groups and one way analysis of variance (ANOVA) and Kruskal-Wallis variance analysis (χ^2) for comparing three groups. Qualitative parameters were compared with (χ^2) and Fisher (p) tests.

Results

Seven patients with primary SS with a mean age of 41.14±16.99 years; 23 patients with secondary SS with a mean age of 52.7±12.1 years; 3 patients with Hodgkin's lymphoma (20.0%); 12 patients

12 pacijenata s non-Hodgkinovim limfomom (NHL) prosječne dobi 45,87±17,36 godina i 15 zdravih pojedinaca prosječne dobi 40,47±13,75 godina. S obzirom na dob nije bilo statistički znatnih razlika među ispitanim skupinama (Kruskal-Wallisov test, χ^2=2,764, p=0,251). Primarna bolest naših pacijenata sa sekundarnim SS-om bila je reumatoidni artritis (RA).

Trajanje tih bolesti, izmjereno u godinama bolovanja od primarnog SS-a, iznosila je 3,29±2,36, sekundarni je SS bio 4,09±2,11, a za oba oblika SS-a iznosio je 3,90±2,16. Nije bilo statistički velikih razlika između tih skupina (Mann-Whitneyev U test, z=0,97, p=0,322).

Srednje vrijednosti za čestoću SCE-a (po metafazi) kod pacijenata sa SS-om (Tablica 1., Slika 1.) te limfomom i kod zdravih sudionika iz kontrolne skupine iznosile su 7,77±1,50, 8,80±0,75 i 6,64±1,56

with non-Hodgkin's lymphoma (NHL) with a mean age of 45.87±17.36 years and 15 healthy individuals with a mean age of 40.47±13.75 years were evaluated. There was no statistically significant difference between these groups in terms of age (Kruskal-Wallis, χ^2=2.764, p=0.251). The primary disease of our patients with secondary SS was rheumatoid arthritis (RA).

Duration of the disease in years of primary SS was 3.29±2.36, of secondary SS was 4.09±2.11 and of all SS was 3.90±2.16. There was no statistically significant difference between these groups (Mann-Whitney U, z=0.97, p=0.322).

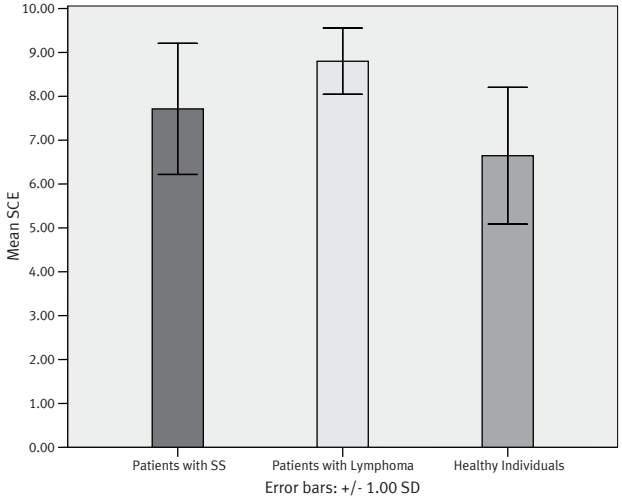
The mean SCE frequencies (per metaphase) in patients with SS (Table 1, Figure 1), lymphoma and healthy controls were 7.77±1.50, 8.80±0.75 and 6.64±1.56, respectively (Figure 2). There were statis-

Tablica 1. Demografski, klinički i laboratorijski podaci i srednje vrijednosti učestalosti SCE-a kod pacijenata sa SS-om po metafazi

Table 1 Demographical, clinical, laboratory information and the mean SCE frequencies of the patients with SS per metaphase.

Broj pacijenta • Patient Number	Spol • Sex	Dob • Age	Tip SS-a • Type of SS	Raspon bolesti • Disease Span	ANA	Anti-Ro/ SS-A	Anti-La/ SS-B	SCE
1.	F	72	SEC./RA	2	+	+	-	8.50
2.	F	67	SEC./RA	6	-	+	-	7.00
3.	F	24	PRIM.	1	+	+	+	6.50
4.	F	54	PRIM.	7	-	+	+	7.70
5.	F	62	SEC./RA	6	-	-	+	8.70
6.	F	48	SEC./RA	3	-	+	+	9.10
7.	F	39	SEC./RA	2	+	+	+	7.70
8.	F	30	SEC./RA	5	+	-	-	7.40
9.	F	70	PRIM.	5	-	+	+	7.90
10.	F	46	SEC./RA	6	+	+	+	8.75
11.	F	65	SEC./RA	4	+	+	+	9.30
12.	F	47	SEC./RA	2	-	+	+	5.80
13.	F	44	SEC./RA	8	-	-	-	12.10
14.	F	58	SEC./RA	5	-	-	-	9.75
15.	F	52	SEC./RA	3	-	+	-	6.60
16.	F	40	SEC./RA	8	-	+	+	8.00
17.	F	41	SEC./RA	8	+	+	-	8.40
18.	F	56	SEC./RA	2	-	-	+	7.70
19.	F	47	SEC./RA	2	-	-	-	8.10
20.	F	39	PRIM.	1	-	+	-	9.60
21.	F	66	SEC./RA	4	-	+	+	4.83
22.	F	23	PRIM.	2	+	+	+	6.00
23.	F	62	SEC./RA	1	-	-	+	7.00
24.	F	32	PRIM.	2	+	+	+	5.90
25.	F	69	SEC./RA	4	+	-	+	6.00
26.	F	47	SEC./RA	3	+	+	-	7.50
27.	F	71	SEC./RA	4	+	-	-	6.40
28.	M	39	SEC./RA	2	-	+	-	9.10
29.	F	44	SEC./RA	4	-	-	+	6.60
30.	F	46	PRIM.	5	+	+	-	7.40

Slika 1. Strelicama je označeno devet razmjena u jednoj metafazi pacijentice sa SS-om
Figure 1 9 exchanges are shown by arrows in a metaphase of a patient with SS



Slika 2. Srednje vrijednosti ± sd SCE-a za svaku skupinu po metafazi
Figure 2 The mean ± sd SCE scores of each group per metaphase

Tablica 2. Pacijenti sa SS-om čija je srednja čestoća SCE-a veća od granične, a pozitivna na ANA Ro. La.
Table 2 Patients with SS whose mean SCE frequencies are higher than the cutting point and ANA. Ro. La positive

Granica • Cutting Point		ANA-Ro-La	ANA-Ro-La	Ukupno • Total
		Sve pozitivno • All positive	Minimalno je jedan negativan • Minimum one is negative	
Manje od 7,225 • Below 7.225	n	3	8	11
	Za granicu • For cutting point	27.3%	72.7%	100.0%
	ANA-Ro-La pozitivno • ANA-Ro-La positive	50.0%	33.3%	36.7%
	Ukupno % • Total %	10.0%	26.7%	36.7%
7,225 i više • 7.225 and above	n	3	16	19
	Za granicu • For cutting point	15.8%	84.2%	100.0%
	ANA-Ro-La pozitivno • ANA-Ro-La positive	50.0%	66.7%	63.3%
	Ukupno % • Total %	10.0%	53.3%	63.3%
Ukupno • Total	n	6	24	30
	Za granicu • For cutting point	20.0%	80.0%	100.0%
	ANA-Ro-La pozitivno • ANA-Ro-La positive	100.0%	100.0%	100.0%
	Ukupno % • Total %	20.0%	80.0%	100.0%

(Slika 2.). Utvrđene su statistički znatne razlike između pacijenata sa SS-om i limfomom (p=0,007) i između pacijenata s limfomom i zdravih iz kontrolne skupine (p=0,0001). Nije bilo statistički velikih razlika između pacijenata sa SS-om i zdravih iz kontrolnih skupina (p=0,107).

Srednja čestoća SCE-a kod pacijenata s primarnim (7,43±1,18) i sekundarnim (7,80±1,59) SS-om bila je slična i zato nije bila uočena statistički znatna razlika između dviju podskupina pacijenata sa SS-om (t- test, t=0,564, p=0,577).

Izračunate su vrijednosti osjetljivosti i specifičnosti (Tablica 2.). Određena je granična vrijednost od 7,225 s najvećom vrijednosti osjetljivosti (0,63) i specifičnosti (0,60).

tically significant differences between patients with SS and lymphoma (p=0.007) and between patients with lymphoma and healthy controls (p=0.0001). There was no statistically significant difference between the patients with SS and the healthy controls (p=0.107).

The mean SCE frequencies of the patients with primary (7.43±1.18) and secondary (7.80±1.59) SS were similar and therefore no statistically significant difference was observed in two subgroups of patients with SS (t- test, t=0.564, p=0.577).

Sensitivity and specificity values were calculated (Table 2). The cut-off point was determined as 7.225 with the highest sensitivity (0.63) and specificity (0.60) value.

Rasprava

Sjögrenov sindrom (SS) jedna je od najčešćih kroničnih sustavnih autoimunih bolesti, a karakterizira ga suhoća sluznica (xerostomia, xerophthalmia, xerotrachea i vaginalna suhoća), otekline na velikim žlijezdama slinovnicama zbog limfoidne infiltracije, atrofični gastritis i non-erozivni poliartritis.

Na to koliko je česta razmjena sestrinskih kromatida mogli bi utjecati mnogobrojni čimbenici poput pušenja, zloporabe opijata, kemoterapeutika, itd. Baš zbog tih čimbenika veliku smo pozornost posvetili izboru sudionika u našim skupinama ispitanika i onih u kontrolnim skupinama. Rowland i suradnici objavili su znatno veće vrijednosti čestoće SCE-a kod pušača i bivših pušača negoli kod nepušača (15). No, zbog difuznog pasivnog pušenja u našoj zemlji, odlučili smo zanemariti taj posljednji parametar. Zatim, poznato je da je SCE često usko vezan za dob. Zato je važno složiti skupine ispitanika i zdravih u kontrolnim skupinama među kojima nema statistički velikih dobnih razlika.

Slavutsky i suradnici dokazali su da se pacijente s Hodgkinovim i non-Hodgkinovim limfomom može procijeniti kao jedinstvenu populaciju s obzirom na analizu SCE-a (16). Zbog malo pacijenata s Hodgkinovim limfomom nije bilo statistički velikih razlika u učestalosti SCE-a između pacijenata s Hodgkinovim (n=3, 20,0 %) i non-Hodgkinovim limfomom (n=12, 80,0 %). Ipak, predlažemo da se te dvije skupine pacijenata s limfomom procjenjuju kao jedinstvena populacija zbog sličnosti čestoće SCE-a kod onih s Hodgkinovim (8,27) i non-Hodgkinovim limfomom (8,92), a i s ukupnim iznosom za limfome (8,80±0,75).

Parkes i njegovi kolege te Privitera i suradnici, istaknuli su da su vrijednosti učestalosti SCE-a za zdrave pojedince, čiji su članovi obitelji imali maligne pojave, statistički više nego kod zdravih pojedinaca bez malignosti u obiteljima (17, 18). Zato smo u našu drugu kontrolnu skupinu uvrstili ispitanike iz zdravih kontrolnih skupina, bez povijesti malignosti kod njihovih prvostupanjskih srodnika.

Srednja vrijednost učestalosti SCE-a za pacijente s limfomom iznosila je 8,80±0,75 po metafazi, a u rasponu od 7,58 do 10,20. Taj je rezultat sličan onima u ostalim studijama koje se bave ispitivanjem učestalosti SCE-a kod pacijenata s limfomom (19-22).

Kliničkim oralnim pregledom svih ispitanika studijskih i kontrolnih skupina ustanovljen je blag ili umjeren oblik parodontne bolesti. Emingil i suradnici istaknuli su da samo agresivne i izrazite pa-

Discussion

Sjögren's syndrome (SS) is one of the most common systemic chronic autoimmune diseases characterized by mucosal dryness (xerostomia, xerophthalmia, xerotrachea and vaginal dryness), major salivary gland enlargement caused by lymphoid infiltration, atrophic gastritis and non-erosive polyarthritis.

Frequency of sister chromatid exchange could be affected by many factors like smoking, drug use, chemotherapeutic agents etc. We paid great attention in choosing our study and control groups because of these factors. Rowland et al. reported that SCE frequencies of the smokers and ex-smokers are remarkably higher than the SCE frequencies of the non-smokers (15). Therefore our study and control groups consisted of patients and people without a history of smoking. But, because of the existence of diffused passive smoking in our country we had to overlook this parameter. Additionally, it is well known that high frequency of SCE is closely related to age. Therefore, it was very important to establish no statistically significant difference between the groups in terms of age.

Slavutsky et al. had shown that patients with Hodgkin's and non-Hodgkin's lymphoma can be evaluated as one population in terms of SCE analysis (16). There was no statistically significant difference in SCE frequencies of the patients with Hodgkin's (n=3, 20.0%) and non-Hodgkin's lymphoma (n=12, 80.0%) because of the low number of patients with Hodgkin's lymphoma. But we suggest also that two groups of the patients with lymphoma can be evaluated as one population because of the similar SCE frequencies of the patients with Hodgkin's (8.27), with non-Hodgkin's lymphoma (8.92) and with lymphoma total (8.80±0.75).

Parkes et al. and Privitera et al. reported that the SCE frequencies of the healthy individuals whose family members developed malignancy are statistically higher than the healthy individuals with no malignancy in their families (17, 18). Therefore, we had formed our second control group from healthy controls with no history of malignancy in their first degree relatives.

The mean SCE frequency for the patients wit lymphoma was found 8.80±0.75 per metaphase and the range was 7.58 to 10.20. This result is similar to the results of the other studies investigating SCE frequency for the patients with lymphoma (19-22).

Oral clinical examination of all the members in the study and control groups revealed mild or mod-

rodontne bolesti mogu utjecati na to koliko se često javlja SCE (23). Zato je taj parametar izostavljen.

Veliku smo pozornost posvetili osnutku naše prve kontrolne skupine u kojoj su bili pacijenti s limfomom čije liječenje još nije počelo zbog poznatih učinaka kemoterapeutika ili citotoksičnih sredstava na čestoću SCE-a, a primjenjuju se u terapiji malignih pojava.

Došli smo do srednje vrijednosti učestalosti SCE-a kod zdravih pojedinaca u iznosu od 6,65±1,56, što je u skladu s prijašnjim studijama (19, 21, 24). Za tri pacijenta (10 %) sa SS-om zabilježili smo srednje vrijednosti učestalosti SCE-a po metafazi veće od vrijednosti izmjerenih kod naše studijske skupine (8,80±0,75) i veće od iznosa za pacijente s limfomom (8,80±0,75). Predviđena pojavnost malignih limfoma u SS-u u literaturi iznosi 2,5 % (25), 3,0 % (10), 5,0 % (11) i 4,3 % (7). Zato pretpostavljamo da ta tri pacijenta čine "rizičnu skupinu za razvoj maligniteta", ako se u obzir uzme samo jedan parametar. No, pažljivi klinički pregledi i pomne kontrole potrebni su kako bi se potvrdili rezultati te studije.

Zahvale

Ovu studiju podupiru rezultati istraživanja Sveučilišta u Istambulu. Broj projekta: T-379 / 08032004.

Ovo je ispitivanje predstavljeno kao poster na Osmom dvogodišnjem kongresu Europske udruge za oralnu medicinu (EOAM-a) od 31. kolovoza do 2.rujna 2006., a održan je u Zagrebu, u Hrvatskoj; broj projekta : UDP – 805 / 05072006.

Ispitivanje je obavljeno na temelju odobrenja Etičkog povjerenstva Medicinskog fakulteta Sveučilišta u Istambulu; broj projekta : 2004 / 778.

erate periodontal diseases. Emingil et al. reported that only aggressive and severe periodontal diseases can effect SCE frequency (23). Therefore this parameter was neglected.

We paid great attention to create our first control group from lymphoma patients whose treatment had not begun because of known effects of chemotherapeutic or cytotoxic agents used in treatment of malignancy to SCE frequency (19, 20, 12).

We found the mean SCE frequency in the healthy individuals to be 6.65±1.56 with accordance in previous studies (19, 21, 24). We observed that the mean SCE frequencies per metaphase of 3 patients (10%) with SS were higher than the mean SCE frequencies per metaphase of our study group (8.80±0.75) and of the patients with lymphoma (8.80±0.75). The estimated prevalence of malignant lymphoma in SS was reported as 2.5% (25), 3.0% (10), 5.0% (11) and 4.3 % (7). Therefore, we hypothesize that these 3 patients can be considered as 'risk group for developing malignancy' if only one parameter is taken into account. But, careful clinical examination and close follow-ups are necessary to verify the results of the present study.

Acknowledgement

This present study was supported by the research found of Istanbul University. Project number: T-379 / 08032004.

This study was presented as poster presentation in the 8th Biennial Congress European Association of Oral Medicine (EAOM) 31 August-2 September, 2006. Zagreb, Croatia; Project number: UDP – 805 / 05072006.

This study was established under approval of the Ethic Committee of Istanbul University, Faculty of Medicine; Project number: 2004 / 778.

Abstract

Purpose: To investigate genomic instability by using Sister Chromatid Exchange (SCE) in the peripheral blood lymphocytes in patients with Sjögren's syndrome (SS). **Patients and Methods:** The frequency of SCE (per metaphase) was investigated in patients with primary Sjögren's Syndrome (n=30), in untreated lymphoma patients (n=15) and healthy individuals (n=15) who constitute the control groups. **Results:** We found the mean frequencies of SCE in the patients with SS, untreated lymphoma and healthy controls as 7.77±1.50, 8.80±0.75, 6.65±1.50, respectively. We found statistically significant differences between patients with SS and lymphoma (p=0.007) and also between patients with lymphoma and healthy controls (p=0.0001). No significant difference was observed between patients with SS and healthy controls in regard of mean frequencies of SCE (per metaphase). **Conclusion:** It is well known that up to 5.0% of all SS patients may develop lymphoma. High frequency of SCE is regarded as a cytogenetic biomarker of early mutagenic effect suggesting increased risk of cancer. We observed that the mean frequencies of sister chromatid exchanges (per metaphase) of three patients with SS were higher than those of untreated lymphoma patients. This result can only be verified by prolonged follow-up of these patients. We suggest that results should be interpreted by evaluating other parameters which play important roles in malignant transformation.

Received: February 14, 2008
Accepted: October 12, 2008

Address for correspondence
Sertan Ergun, D.D.S, Ph.D.
Istanbul University, Faculty of Dentistry,
Department of Oral Medicine and Surgery,
34393 Capa, Istanbul, Turkey
sertanergun@gmail.com

Key words
Sjögren's Syndrome, Sister Chromatid Exchange, Lymphoma

References

1. Abraham CM, al-Hashimi I, Haghighat N. Evaluation of the levels of oral Candida in patients with Sjögren's syndrome. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1998;86(1):65-8.
2. Al-Hashimi I, Haghighat N, Fox PC. Salivary electrophoresis in the diagnosis of Sjögren's syndrome. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1998;85(5):542-7.
3. Carpenter GH, Proctor GB, Pankhurst CL, O'Donohue J, Scott D, Hunnable MP. Sialochemical markers of salivary gland involvement with Sjögren's syndrome secondary to rheumatoid arthritis and primary biliary cirrhosis. J Oral Pathol Med. 2000;29(9):452-9.
4. Kapsogeorgou EK, Dimitriou ID, Abu-Helu RF, Moutsopoulos HM, Manoussakis MN. Activation of epithelial and myoepithelial cells in the salivary glands of patients with Sjögren's syndrome: high expression of intercellular adhesion molecule-1 (ICAM.1) in biopsy specimens and cultured cells. Clin Exp Immunol. 2001;124(1):126-33.
5. Kindelan SA, Yeoman CM, Douglas CW, Franklin C. A comparison of intraoral Candida carriage in Sjögren's syndrome patients with healthy xerostomic controls. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1998;85(2):162-7.
6. Kobayashi H, Ohara I, Kanoh S, Motoyoshi K, Aida S, Kohyama N. Clinicopathological features of pure mica pneumoconiosis associated with Sjögren syndrome. Am J Ind Med. 2004;45(3):246-50.
7. Yamamoto K. Pathogenesis of Sjögren's syndrome. Autoimmun Rev. 2003;2(1):13-8.
8. Masaki Y, Sugai S. Lymphoproliferative disorders in Sjögren's syndrome. Autoimmun Rev. 2004;3(3):175-82.
9. Franceschini F, Cavazzana I. Anti-Ro/SSA and La/SSB antibodies. Autoimmunity. 2005;38(1):55-63.
10. Kamalı S, Kasapoglu E, Cefle A, Sayarlıoğlu M, Ocal L, Gül A et al. Clinical and laboratory characteristics of 35 patients with primary Sjogren Syndrome: Experience of the Istanbul University, Department of Internal Medicine, Division of Rheumotology. Istanbul Tip Fak Mecmuasi. 2004;67(2);104-8.
11. Hakulinen T, Isomaki H, Knekt P. Rheumatoid arthritis and cancer studies based on linking nationwide registries in Finland. Am J Med. 1985;78(1):29-32.
12. Oztürk S, Palanduz S, Karan MA, Tascıoglu C, Guler K, Ertan N et al. Investigation of the effect of Interferon α 2a on human chromosomes in vitro by the use of sister chromatid exchange. Istanbul Tip Fak Mecmuasi. 1998;61(1);29-33.
13. Vitali C, Moutsopoulos HM, Bombardieri S. The European Community Study Group on diagnostic criteria for Sjögren's syndrome. Sensitivity and specificity of tests for ocular and oral involvement in Sjögren's syndrome. Ann Rheum Dis. 1994;53(10):637-47.
14. Moorhead PS, Nowell PC, Mellman WJ, Battips DM, Hungerford DA. Chromosome preparations of leukocytes cultured from human peripheral blood. Exp Cell Res. 1960;20:613-6.
15. Rowland RE, Harding KM. Increased sister chromatid exchange in the peripheral blood lymphocytes of young women who smoke cigarettes. Hereditas. 1999;131(2):143-6.
16. Slavutsky I, de Vinuesa ML, Larripa I, de Pargament MM, de Salum SB. Sister chromatid exchange in malignant lymphomas. Cancer Genet Cytogenet. 1984;13(2):153-8.
17. Ghidoni A, Privitera E, Raimondi E, Rovini D, Illeni MT, Cascinelli N. Sister chromatid exchange in phytohemagglutinin-stimulated lymphocytes of nonfamilial cutaneous malignant melanoma patients. Basic Life Sci. 1984;29 Pt B:855-8.
18. Parkes DJ, Scott D, Stewart A. Changes in spontaneous SCE frequencies as a function of sampling time in lymphocytes from normal donors and cancer patients. Mutat Res. 1985;147(3):113-22.
19. Bilban-Jakopin C, Bilban M. Genotoxic effects of radiotherapy and chemotherapy on circulating lymphocytes in patients with Hodgkin's disease. Mutat Res. 2001;497(1-2):81-8.
20. Brown T, Dawson AA, King DJ, Bullock I, Watt JL. Variation of SCE frequency in lymphocyte cultures from patients with Hodgkin's disease before, during, and after treatment. Cancer Genet Cytogenet. 1986;20(1-2):53-61.
21. Caggana M, Kelsey KT. Sister chromatid exchange frequency in Hodgkin's disease patients with elevated in vivo hprt mutant frequencies. Carcinogenesis. 1991;12(11):2141-5.
22. Peterseim UM, Küster W, Gebauer HJ, Meschig R, Plewig G. Cytogenetic effects during extracorporeal photopheresis treatment of two patients with cutaneous T-cell lymphoma. Arch Dermatol Res. 1991;283(2):81-5.
23. Emingil G, Sapmaz G, Biçakçi N, Ozkinay F. Sister chromatid exchange (SCE) analysis in periodontitis. J Clin Periodontol. 2002;29(9):811-5.
24. Lioi MB, Santoro A, Barbieri R, Salzano S, Ursini MV. Ochratoxin A and zearalenone: a comparative study on genotoxic effects and cell death induced in bovine lymphocytes. Mutat Res. 2004;557(1):19-27.
25. Liu BH, Yu FY, Wu TS, Li SY, Su MC, Wang MC et al. Evaluation of genotoxic risk and oxidative DNA damage in mammalian cells exposed to mycotoxins, patulin and citrinin. Toxicol Appl Pharmacol. 2003;191(3):255-63.
26. Rencüzogullari E, Ila HB, Kayraldiz A, Topaktaş M. Chromosome aberrations and sister chromatid exchanges in cultured human lymphocytes treated with sodium metabisulfite, a food preservative. Mutat Res. 2001;490(2):107-12.
27. Somer BG, Tsai DE, Downs L, Weinstein B, Schuster SJ; American College of Rheumatology ad hoc Committee on Immunologic Testing Guidelines. Improvement in Sjögren's syndrome following therapy with rituximab for marginal zone lymphoma. Arthritis Rheum. 2003;49(3):394-8.