ORİJİNAL ARAŞTIRMA ORIGINAL RESEARCH

Genomic Damage in Patients with Chronic Renal Failure

Kronik Böbrek Yetmezliği Olan Hastalarda Genomik Hasar

Ali KARAMAN, MD,[a]
Figen KESKİNLER, MD[b]

[a]Department of Medical Genetics,
[b]Hemodialysis Unit,
Erzurum State Hospital, Erzurum

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Yazışma Adresi/*Correspondence:*
Ali KARAMAN, MD
Erzurum State Hospital,
Department of Medical Genetics,
Erzurum,
TÜRKİYE/TURKEY
alikaramandr@hotmail.com

ABSTRACT Objective: Chronic renal failure (CRF) is associated with a high incidence of cancer. The frequencies of sister chromatid exchange (SCE) and micronucleus (MN) in peripheral blood lymphocytes (PBL) is extensively used as biomarkers of chromosomal damage and genome stability in human populations. The aim of this study was to determine the SCE and MN rates of the PBL in patients with chronic renal failure (CRF). **Material and Methods:** This study was conducted between February 2005 and June 2006 in the Erzurum State Hospital. We analyzed lymphocytes from 34 (12 females and 22 males) CRF patients and 30 (14 females and 16 males) normal controls for SCE and MN frequencies. In the patient group, the mean dialytic age was 3.23 ± 1.56 years (range 1 to 6 years). **Results:** SCE rate was significantly increased in CRF patients (9.48 ± 1.23) compared with controls (4.85 ± 0.81) (p< 0.001). Similarly, MN incidence was significantly higher in CRF patients (25.82 ± 7.03) compared with controls (12.79 ± 4.62) (p< 0.001). The SCE and MN rates positively correlated with creatinin levels (r= 0.906; p< 0.001 and r= 0.534; p<0.01, respectively). However, the SCE and the MN rates did not correlate with the blood levels of urea (r= 0.427, p> 0.05 and r= 0.409, p> 0.05, respectively). **Conclusion:** Our results suggest that high SCE and MN frequencies reflect increased DNA damage in CRF patients and this may contribute to the increased incidence of many chronic diseases in such patients.

Key Words: Micronucleus, chromosome-defective; sister chromatid exchange; renal failure

ÖZET Amaç: Kronik böbrek yetmezliği (KBY) kanser insidansında artışa neden olur. Periferik kan lenfositleri (PKL)'nde kardeş kromatit değişimi (KKD) ve mikronükleus (MN) sıklığı, insan popülasyonlarında kromozomal hasarın ve genom stabilitesinin bir göstergesi olarak yaygın kullanılmaktadır. Bu çalışmada amaç, KBY olan 34 hastada PKL'de KKD ve MN sıklığını tespit etmektir. **Gereç ve Yöntemler:** Bu çalışma, Şubat 2004 ve Haziran 2006 tarihleri arasında Erzurum Devlet Hastanesi'nde yapıldı. KKD ve MN sıklığı için, 34 (12 kadın ve 22 erkek) KBY hastası ve 30 (14 kadın ve 16 erkek) sağlıklı kontrol incelendi. Hasta grubunun ortalama diyaliz süresi 3.23 ± 1.56 yıl idi (1-6 yıl). **Bulgular:** KKD oranı KBY olan hastalarda (9.48 ± 1.23) kontrollere (4.85 ± 0.81) göre belirgin olarak daha yüksekti (p< 0.001). Benzer şekilde, MN insidansı da KBY olan hastalarda (25.82 ± 7.03) kontrollere (12.79 ± 4.62) görece belirgin olarak daha yüksekti (p< 0.001). KKD ve MN oranları ile kreatinin düzeyi pozitif bir ilişki sergilemekteydi (sırasıyla r=0.906; p< 0.001 ve r= 0.534; p< 0.01). Ancak, KKD ve MN oranları, kan üre seviyesi ile ilişkili değildi (sırasıyla r= 0.427, p> 0.05 ve r= 0.409, p> 0.05). **Sonuç:** Elde ettiğimiz bulgular, yüksek KKD ve MN sıklığının, KBY olan hastalarda DNA hasarındaki artışın göstergesi olduğunu ortaya koymaktadır; bu bulgular, bu hastalarda pek çok kronik hastalığın insidansındaki artışın açıklanmasına katkı sağlayabilir.

Anahtar Kelimeler: Mikronükleus; defektif kromozom; kardeş kromatid değişimi; böbrek yetmezliği

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Chronic renal failure (CRF) is associated with a high incidence of cancer.[1,2] Lifestyle factors, environmental exposures and genetic background interact in the development of many cancer types.

Several pathogenic mechanisms were suggested to explain this such as microinflammation and oxidative stress, which involve the whole cell structure (proteins, membrane lipids, carbonhydrates and DNA).[3-5] Great interest has recently been paid to oxidative DNA damage because of its genetic consequences, linked to early aging, neurodegenerative diseases, diabetes mellitus, atherosclerosis, mutagenesis and carcinogenesis.[6-8]

The genome damage in the lymphocytes of peripheral blood has been widely used as a biomarker of carcinogenesis from genotoxic environmental factors, and long-term studies have demonstrated its validity and high clinical predictivity.[9]

The SCE phenomenon is widely used as a reliable and sensitive indicator of chromosome (DNA) instability, since the SCE patterns can reveal a general genome instability.[10] Variation in DNA repair mechanisms or detoxifying enzymes have been implicated as the cause of genetic susceptibility associated with many chronic diseases.[11] SCE in peripheral lymphocytes has been widely used in assessing exposure to chemicals for their possible genotoxic potential.[12,13]

Micronuclei are DNA-containing structures, which are formed during mitosis and result from chromosomal breaks or from whole chromosomes incorrectly distributed during mitosis. They represent a subgroup of all chromosomal aberrations. The MN frequency test, widely accepted for in vitro and in vivo genotoxicity investigations, is a sensitive marker of genomic damage.[14,15]

Patients with CRF and patients undergoing maintenance dialysis have an increased risk of developing cancer compared with the general population.[2] This risk is increased for several reasons, including a weakened immune system, impaired DNA repair mechanisms, chronic infections and inflammation, reduced antioxidant defense, and the accumulation of uremic toxins.[16-19]

The uremic milieu, with increased oxidative stress and uremic toxins, causes severe damage to DNA. This was shown for mitochondrial DNA, which contains a higher frequency of deletions in patients with CRF than that in control subjects.[20]

In patients with CRF the presence of massive genome damage has repeatedly been demonstrated using different methodologies, including the frequency of micronuclei in peripheral blood lymphocytes (PBL) and the evaluation of the SCE rate.[21-26] However, this kind of genome damage in patients with CRF is still a controversial issue. Therefore, in this study, we aimed to confirm, by assessing SCE and micronucleus (MN) frequencies, whether genetic impairment and DNA damage had an effect on the evil progression of chronic renal failure.

MATERIAL AND METHODS

This study was conducted between February 2005 and June 2006 in the Erzurum State Hospital. We performed SCE and MN analysis in 34 (12 females and 22 males) patients with CRF (mean age: 50.05± 17.83 years) and in 30 (14 females and 16 males) healthy controls (mean age: 47.21 ± 11.65 years). We calculated the sample size with the following formula: $n= \{Nt^2s^2\} \text{¸} \{d^2 (N-1)+t^2s^2\}$; N =150, t =1.96, s =3, d =1, n =28.24. The age of the patients ranged 18-73 years, and the age of the controls were 20-70 years. The primary renal diseases were as follows: chronic glomerulonephritis (n= 13), diabetic nephropathy (n= 8), polycystic kidney disease (n=2), primary interstitial nephritis (n= 5), and nephrovascular disease (n= 6). In the patient group, the mean dialytic age was 3.23 ± 1.56 years (range 1-6 years). All patients underwent maintenance hemodialysis three times weekly and used multiple vitamin preparations. The patients had not received chemotherapy or radiotherapy during the last 6 months. They were selected among non-smoking and nonalcoholic subjects. No subject had a history of viral infection, bacterial infection and radiation exposure. The patient and control groups were selected for their similar habits. The hospital Ethical Committee approved the human study. We obtained written informed consent from each participant. All patients were analyzed prior to hemodialysis therapy. For SCE and MN analysis, 3 mL of heparinized blood was drawn from each individual.

SISTER CHROMATID EXCHANGE ANALYSIS

Cultures were established by adding 0.5 mL of blood to 5 mL karyotyping medium (Biological Industries, Beit Haemek, Israel) with 2% phytohaemagglutinin M (PHA) (Biological Industries) and incubating for 24 h at 37°C. A 5-bromo-2'-deoxyuridine (BrdU) (Sigma, USA) solution at a final concentration of 5μg/mL was added. Lymphocytes were cultured in the dark for 48 h and metaphases were blocked during the last 2 h with colcemid (Biological Industries) at a final concentration of 0.1 μg/mL. Further processing included hypotonic treatment, fixation, slide preparation and fluorescein plus Giemsa (FPG) staining for detection of SCE.[27] Fifty second-division metaphases were scored on coded slides by a single observer as the number of SCE/cell per subject.

MICRONUCLEI IN PERIPHERAL LYMPHOCYTES

Cultures were established adding 0.5 mL blood to 5 mL karyotyping medium (Biological Industries) with 2% PHA (Biological industries) and incubating for 44h at 37°C. Cytochalasin B (Sigma, USA) was added at a final concentration of 6mg/ml to induce binuclear cell formation, and the culture was kept at 37°C for 72h. The cells were then treated hypotonically with 0.0075M KCl for 5 min at room temperature and were fixed in methanol/acetic acid (3:1). Cells were dropped onto slides and were stained with 5% Giemsa in phosphate buffer (pH 6.8) for 5 min. About 1000 binucleated cells from each case were examined for micronuclei by an experienced observer.[28]

STATISTICAL ANALYSIS

The SCE and MN data were analyzed statistically by the Mann-Whitney U-test. Creatinin, and blood urea nitrogen (BUN) levels were analyzed statistically by Student's *t*-test. To evaluate the correlations between the duration of renal failure (dialytic age), type of renal disease, age, sex, creatinin and BUN levels, SCE frequency and MN rate, the coefficients of Pearson's correlation were calculated. A P value less than 0.05 was considered significant. Statistical analysis was performed with SPSS 11.5 Package (SPSS Inc., IL, USA).

RESULTS

The frequencies of SCE and MN and the characteristics of patients were shown in Table 1. The mean SCE/cell frequency in the patient group (9.48 ± 1.23; range 7.65 to 12.40) was significantly higher than in the control group (4.85 ± 0.81; range 3.50 to 6.40) (p< 0.001; Mann-Whitney *U* test). Similarly, the mean MN rate /1.000 binucleated (BN) cells in the patient group (25.82 ± 7.03; range 12 to 42) was significantly higher than in the control group (12.79 ± 4.62; range 8 to 23) (p< 0.001; Mann-Whitney *U* test). The serum creatinin and BUN levels were summarized in Table 2. The BUN levels of the patients ranged from 110 to 372 mg/dL (mean ± SD =232.2 ± 103.56 mg/dL) and creatinin from 2.31 to 17.6 mg/dL (mean ± SD = 9.56 ± 4.07 mg/dL). Significantly increased levels of serum creatinin and BUN levels were noted in patients with CRF (Student's *t*-test). The SCE levels and MN frequencies positively correlated [r= 0.906; p< 0.001 and r= 0.534; p< 0.01, respectively Pearson's correlation coefficient (r)] with serum creatinin concentrations. On the other hand, the SCE frequency did not correlate with age, sex, BUN level, type of renal disease or duration of renal disease (dialytic age) (r= 0.012, p> 0.05; r= 0.025,p> 0.05; r= 0.427, p> 0.05; r= 0.138, p> 0.05; r= 0.116, p> 0.05).

TABLE 1: Sister chromatid exchange (SCE) and micronucleus (MN) frequencies in lymphocytes of chronic renal failure patients and controls.

	Sex	Age (years)	Dialytic Age (years)	SCE	MN/1.000 BN
Group	F/M	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Patients (n= 34)	12/22	50.05 ± 17.83	3.23 ± 1.56	9.48 ± 1.23	25.82 ± 7.03
Controls(n= 30)	14/16	47.21 ± 11.65	-	4.85 ± 0.81	12.79 ± 4.62
P-value	-	-	-	p< 0.001	p< 0.001

BN: Binucleated SCE: Sister chromatid exchange, MN: Micronucleus.

TABLE 2: Means of serum creatinin and blood urea nitrogen (BUN) levels in patients and controls.

Group	Creatinine (mg/dL) Mean ± SD	BUN (mg/dL) Mean ± SD
Patients (n= 34)	9.56 ± 4.07	232.2 ± 103.56
Controls (n= 30)	1.02 ± 0.29	21.93 ± 8.17
P-value	p<0.01	p<0.01

BUN: blood urea nitrogen.

Similarly, the MN rate did not correlate with age, sex, BUN level, type of renal disease or duration of renal failure (dialytic age) (r= 0.015, p> 0.05; r= 0.042, p> 0.05; r= 0.409, p> 0.05; r= 0.134, p> 0.05; r= 0.123, p> 0.05).

DISCUSSION

Exposure of cells to a variety of genotoxic and cytotoxic agents have the potential to elicit prolonged and dynamic changes that compromise the stability of the cellular genome.[29-31] In recent studies, enhanced genomic damage in peripheral blood lymphocytes of uremic patients was observed.[8,26] Many of these changes, whether induced directly or indirectly by DNA damage, lead to increases in gene mutation and amplification, reduced cloning efficiency, elevated micronuclei, sister chromatid exchanges, and multiple kariotypic abnormalities.[32]

Cytogenetic tests have been widely used in medicine for the assessment of a causal association between disease and cytogenetic damage. SCE, as an indicator of DNA damage, might reflect an instability of DNA or deficiency of DNA repair. Therefore, it could be used to investigate any causal association between various diseases and any cytogenetic damage.[33-35]

SCE is known to be increased by exposure to various genotoxic materials, and seems to reflect the repair of DNA lesions by homologous recombination.[36,37] Important sources of exposure include diet, general environment, medical exposure to ionizing radiation, and internal generation of genotoxic species. Internal phenomena such as metabolism, errors of DNA replication, inflammation, and oxidative stress may be of importance. Inflammatory diseases, oxidative stress, and radiation exposure have been associated with the generation of clastogenic factors, which may be quite persistent, might play an important part in carcinogenesis.[38,39]

MN is a sensitive indicator of exogenously or endogenously caused genetic damage and has become an important endpoint in genotoxicity testing both in vivo and in vitro.[12,40] Increased MN frequencies have been reported in the lymphocytes of smokers and chimney sweeps with elevated aromatic DNA adducts and cancer patients treated with irradiation or genotoxic chemotherapeutics.[41-44]

Our study, which showed increased frequencies of SCE and MN in lymphocytes of the CRF patients, could support these observations, since induction of changes in the DNA that lead to mutations may play a role in carcinogenicity. The SCE and MN frequencies in patients with CRF correlated with serum creatinin concentrations. However, the SCE and MN rates did not correlate with the blood levels of urea. These results are in line with the impaired DNA repair shown previously in uremic patients.[25,26,45] As already stated in the literature, end-stage renal disease patients are particularly exposed to oxidative damage.[3-5,46] In addition to the impairment of DNA repair, the increased formation of reactive oxygen species (ROS) due to frequent chronic infections and/or bioincompatibility reactions with the dialysis membranes, as well as the shown reduced antioxidant defense in these patients, also may have contributed to the increased chromosome damage.[47-50]

Uremic toxins by definition are capable of interacting with biological systems and produce a deleterious biological response.[51] This condition of chronic inflammation in the kidney as well as the chronic uremic state may be reflected by a systemic chronic alteration of the immune system, eg, increased SCE rate in uremic patients, and elevated MN rate in peripheral lymphocytes.[25,26,45,52]

It is now widely accepted that in patients with end-stage renal disease, immunodeficiency and an increased predisposition to cancer deve-

lopment coexist. Both of these conditions can be related to the mutagenic action of physical, chemical and biological agents which end-stage renal disease patients are exposed to because of the inadequate clearance of endogenous substances and the contact with materials included in the dialysis circuit.[43,48-50]

In conclusion, our results suggest that increased genomic instability may be associated with CRF. Thus, the increased SCE and MN frequencies and the impaired DNA repair in uremia may be related to such toxic substances and may account for the higher incidence of many chronic diseases in this condition.

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