Therapeutic effects of statins on chromosomal DNA damage of dyslipidemic patients

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Impact statement

In literature, it is possible to find some *in vitro* cytokinesis-block micronucleus (CBMN) assay studies about human lymphocytes and statins. But, there are no data on CBMN-cytome (CBMN-cyt) assay parameters related to statin therapy in patients with dyslipidemia. The present study is the first to evaluate CBMN-cyt assay biomarkers and 8-OHdG levels in patients with dyslipidemia before treatment and after treatment with statins (5–10 mg/day rosuvastatin or 10–20 mg/day atorvastatin). In this study we show that statin therapy decreased chromosomal DNA damage (micronuclei and nucleoplasmic bridges) and nuclear division index (NDI) values in patients with dyslipidemia by possible molecular reasons independent of oxidative DNA damage. In addition, the decrease of chromosomal DNA damage and NDI values with statin treatment could be indicated by the association between statin use and reduced risk of cancer.

Abstract

Statins are a group of cholesterol lowering drugs and frequently used in the therapy of dyslipidemia. Our knowledge of the impact of statin therapy on DNA damage is as yet rudimentary. In this study, we aimed to assess the possible (1) genotoxic, cytostatic, and cytotoxic effects of statins in peripheral blood lymphocytes by using the cytokinesis-block micronucleus cytome (CBMN-cyt) assay, and (2) oxidative DNA damage by measuring plasma 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels in response to statin therapy. Thirty patients with dyslipidemia who had no chronic diseases and did not use any medicines that interfere lipid values and twenty control subjects were included in the study. Statin therapy was initiated at risk-stratified doses. Blood samples were taken before and after treatment with statins and from control subjects, and CBMN-cyt assay parameters and 8-OHdG levels were evaluated. The chromosomal DNA damage (micronuclei and nucleoplasmic bridges [NPBs]), cytostasis (nuclear division index [NDI]), and cytotoxicity (apoptotic and necrotic cell frequencies) were decreased in patients with dyslipidemia after statin treatment. No significant differences were found for 8-OHdG levels between patients with dyslipidemia before or after statin therapy. The total cholesterol and low-density lipoprotein-cholesterol levels showed positive correlations with NPB frequency in patients with dyslipidemia prior to statin treatment. The present study is the first to evaluate CBMN-cyt assay biomarkers and 8-OHdG levels in patients with dyslipidemia before and after treatment with statins. The observed reductions of chromosomal DNA damage and NDI values with statin treatment could represent an important and under-appreciated pleiotropic effect of these agents.

Keywords: Dyslipidemia, DNA damage, cytokinesis-block micronucleus cytome assay, micronucleus, statin therapy, 8-hydroxy-2′-deoxyguanosine levels

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Introduction

Dyslipidemia is a major risk factor in the development of atherosclerotic cardiovascular disease (ASCVD). Treatment of dyslipidemia is known to reduce the morbidity and mortality of ASCVD.[1,2] The patient-centered management of dyslipidemia addresses lifestyle modification and the initiation of drug therapies when appropriate.[3,4] Lifestyle changes such as diet, exercise, weight loss, and smoking cessation to improve cardiovascular health and to lower low-density lipoprotein (LDL) cholesterol have been recommended for all patients. However, these may sometimes be inadequate and statins are used to inhibit 3-hydroxy-3-methyl-glutaryl coenzyme A reductase and reduce LDL-cholesterol.[1,4]

The cytokinesis-block micronucleus cytome (CBMN-cyt) assay is a genotoxicity method used to cytologically evaluate genome damage (presence of micronuclei [MN], nucleoplasmic bridges [NPBs], and nuclear buds [NBUDs]), cytostasis (the proportion of mononucleated, binucleated (BN), multinucleated cells; nuclear division index [NDI]), and cytotoxicity (necrosis to apoptosis cell ratios) in cultured human and/or mammalian cells.[5–7] A micronucleus represents a chromosome or chromosomal fragment that was not transferred into daughter nuclei during mitosis. It is a marker of chromosomal instability. NPBs develop from dicentric chromosomes, which can be a manifestation of incorrectly repaired DNA breaks, telomere end fusion, or defective segregation of sister chromatids during anaphase. NBUDs are comprised of amplified DNA and DNA repair complexes.[8]

Oxidative DNA damage plays an important role in the progress of a lot of diseases, including cancer and aging. 8-hydroxy-2′-deoxyguanosine (8-OHdG) is a product of DNA base modification generated by the oxidation of deoxyguanosine. It is one of the most sensitive biomarkers of oxidative DNA damage and oxidative stress and can be detected in urine, plasma, and serum.[9]

Oxidative stress and oxidative DNA damage may contribute to the histologic injury accompanying dyslipidemia. There are a few studies reporting oxidative DNA damage in the setting of hyperlipidemia using single-cell gel electrophoresis (comet assay).[10,11] Harangi *et al.*[10] showed increased DNA damage in hyperlipidemic subjects. But Pereira *et al.*[11] reported that no significant differences were observed for oxidative DNA damage (DNA strand breakage) between hypercholesterolemic and control subjects. On the other hand, several studies have shown that statins decreased oxidative DNA damage in the leukocytes of patients with diabetic dyslipidemia.[12–14] In our previous studies, we have demonstrated that genomic damage is enhanced in obesity, nodular thyroid, and some pituitary diseases.[15–20]

There are no data available in the literature about plasma 8-OHdG levels and CBMN-cyt assay biomarkers in the lymphocytes of patients with dyslipidemia related to statin therapy, although there are a limited number of studies on the association between oxidative DNA damage and dyslipidemia. Thus, in this study, we aimed to assess possible genotoxic, cytostatic, and cytotoxic effects in peripheral blood lymphocytes by using CBMN-cyt assay and oxidative DNA damage by using the plasma 8-OHdG levels of statin therapy and also their relationship with lipid levels of patients with dyslipidemia who had no current endocrine disease (thyroid disease and diabetes mellitus), hypertension, or cardiovascular disease.

Materials and methods

Subjects

The study included 30 patients with previously untreated dyslipidemia (23 female and 7 male; median age of 51.5, ranging from 27 to 73 years), who did not have any endocrine disease (thyroid disease and diabetes mellitus), hypertension, or cardiovascular disease and did not use any medicines that interfere with lipid values, who were admitted to the Department of Endocrinology at Erciyes University's Medical Faculty from April 2014 to December 2016. An acceptable dose of standard statin therapy (5–10 mg/day rosuvastatin or 10–20 mg/day atorvastatin) was started according to the lipid levels and characteristics of the patients.[21] Duration of treatment was between 3 and 10 months (median duration of 4.5 months). Twenty healthy control subjects (14 female and 6 male; median age of 46.5, ranging from 31 to 70 years) of similar age and sex to patients were recruited in our study. Excluded from the study were all participants who had any cancer, viral infection, inflammatory disease, a history of exposure to various agents that may alter CBMN-cyt assay parameters and 8-OHdG levels.[16] All subjects provided written informed consent after the purpose and nature of the study had been explained. The study was approved by Erciyes University Medical Faculty Research Ethics Committee (No. 2014/185) and conducted in accordance with the Declaration of Helsinki and local laws, which afforded greater protection to the participants.

Heights (m), weights (kg), abdominal circumferences (cm), hip circumferences (cm), and waist circumferences (cm) of each participant were measured. Body mass index (BMI) was calculated by dividing the weight (kg) by the height in meters squared. The waist-to-hip ratio (WHR) was calculated as the waist measurement divided by the hip measurement.

Blood samples of patients with dyslipidemia before and after treatment with statins and of control subjects were taken after a 12 h overnight fast for the evaluation for measurement of fasting glucose, triglycerides, total cholesterol, LDL-cholesterol, HDL-cholesterol, aspartate aminotransferase (AST), and alanine aminotransferase (ALT).

The diagnosis of dyslipidemia was made according to the criteria of $\geq$190 mg/dL of LDL-cholesterol concentrations or $\geq$160 mg/dL of LDL-cholesterol concentrations in patients with high risk (genetic evidence, etc.) by two measurements.[21]

Whole blood cultures of human lymphocytes

Heparinized whole blood samples ($\sim$4 mL) were taken from patients with dyslipidemia before and after treatment with statins and from control subjects matched.

Approximately 0.4 mL of blood samples were cultivated in 5 mL of peripheral blood karyotyping medium, supplemented with 2% phytohaemagglutinin-M, by duplicate cultures to determine intra-individual differences (all sourced from Biological Industries, Kibbutz Beit Haemek, Israel).[7,16]

The CBMN-cyt assay

The CBMN-cyt assay was carried out as described by Fenech[6,7] with some modifications. Briefly, 3 μg/mL of cytochalasin-B (Sigma-Aldrich, St Louis, MO) was added to cultures at 44 h. After cultivation for 72 h, the cells were treated with hypotonic solution, fixed, spread onto slides and stained with Giemsa (Merck).[7,15,16,22] The different slides of the duplicate cultures for each patient before and after treatment with statins and control subject were analyzed. A score was obtained from two different scorers by using a Zeiss light optical microscope. For the selection of BN cells and identification of CBMN-cyt assay parameters, previously published criteria were followed.[7,15–17] One thousand BN cells were scored from each patient before and after treatment with statins and control subject and the number of MN, NPBs, and NBUDs was recorded to determine chromosomal DNA damage. The number of necrotic and apoptotic cells was recorded in 1000 viable mononucleated cells to determine cytotoxicity.[7,15–17,22]

The number of bi-, tri-, and tetra-nucleated cells was also scored in 1000 viable mononucleated cells to determine cytostasis. NDI values were calculated using the formula of $NDI = (M1 + 2M2 + 3M3 + 4M4)/N$.[7,15–17,22,23]

Determination of 8-OHdG levels

To determine 8-OHdG levels, plasma was extracted from heparinized blood samples (2–3 mL) of each patient with dyslipidemia before and after treatment with statins and control subject by centrifugation for 15 min at 3000 rpm and room temperature and stored at −80°C. Before starting the ELISA assay analysis, the samples were ultrafiltrated using a filter of 10,000 molecular weight cut off to separate interfering substances from plasma (Amicon Ultra-0.5 Centrifugal Filter Devices, Millipore, Ireland). Then, 8-OHdG levels in plasma samples were measured using an ELISA assay kit (NWK-8-OHdG02, Northwest Life Science Specialties, LLC, Vancouver, USA). Plasma 8-OHdG levels were shown in ng/mL. Calibration, curve fitting, and data analysis were performed by manufacturer's guidelines.[15–17]

Statistical analysis

All statistical analyses were performed using Statistical Package for Social Sciences (SPSS) software (version 15, SPSS, Inc., Chicago, IL, USA). Before statistical analysis of data, the data distributions for normality were evaluated by Shapiro–Wilk test, histogram, and Q-Q plot. The CBMN-cyt assay parameters, plasma 8-OHdG levels, and basic characteristics (age, BMI, WHR, serum fasting glucose, triglycerides, total cholesterol, LDL-cholesterol, HDL-cholesterol, AST, ALT) in patients with dyslipidemia were tested with the Wilcoxon of two dependent samples tests between before and after treatment with statins and with the Mann–Whitney U of two independent samples tests between before treatment patients and control subjects and after treatment patients and control subjects. Possible relationships between CBMN-cyt assay parameters, plasma 8-OHdG levels, age, and basic characteristics were evaluated using Spearman's rho correlation analysis. p values of < 0.05 were considered statistically significant.

Results

Table 1 shows median (min–max) values for basic characteristics of patients with dyslipidemia before and after treatment with statins and of control subjects. No statistically significant differences of WHR, serum fasting glucose, AST, and ALT were found between values before and after treatment with statins in patients with dyslipidemia ($p > 0.05$). After treatment with statins, the significant decreases in BMI, serum triglycerides, total cholesterol, and LDL-cholesterol levels and a significant increase in serum HDL-cholesterol levels were observed in patients with dyslipidemia. There were significant differences in BMI, serum fasting glucose, triglycerides, total cholesterol,

Table 1. Basic characteristics of patients with dyslipidemia before and after treatment with statins and of control subjects. Data are given as median (min–max).

	Control subjects (n = 20)	Before treatment with statins (n=30)	After treatment with statins (n=30)	P value[a]	P value[b]	P value[c]
BMI (kg/m^2)	24.00 (20.00–27.00)	28.56 (22.00–40.40)	28.11 (22.40–39.95)	<0.001	<0.001	<0.05
WHR	0.80 (0.70–1.10)	0.87 (0.76–1.10)	0.88 (0.75–1.13)	0.160	0.128	0.944
Fasting glucose (mg/dL)	83.50 (71.00–115.00)	97.00 (83.00–142.00)	98.00 (85.00–131.00)	<0.001	<0.001	0.688
Triglycerides (mg/dL)	98.00 (35.00–390.00)	147.55 (55.00–619.00)	112.00 (50.10–435.50)	<0.05	0.247	<0.001
Total cholesterol (mg/dL)	165.50 (145.00–265.00)	290.35 (177.00–461.00)	192.25 (136.00–302.00)	<0.001	<0.05	<0.001
LDL-cholesterol (mg/dL)	97.10 (32.89–177.50)	196.71 (67.00–380.40)	110.60 (64.80–231.60)	<0.001	0.406	<0.001
HDL-cholesterol (mg/dL)	46.65 (29.30–85.60)	49.00 (26.00–95.30)	54.20 (29.30–84.00)	0.620	<0.05	<0.01
AST (u/L)	24.00 (10.00–47.00)	17.00 (10.60–23.00)	17.00 (6.00–38.90)	<0.001	<0.01	0.577
ALT (u/L)	21.00 (7.00–52.00)	16.00 (9.80–39.00)	17.00 (9.00–45.40)	0.207	0.097	0.527

ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: body mass index; HDL: high-density lipoprotein; LDL: low-density lipoprotein; WHR: waist-to-hip ratio.

[a]p value when the comparisons are between the control and before treatment with statin values (Mann–Whitney U test).

[b]p value when the comparisons are between the control and after treatment with statin values (Mann–Whitney U test).

[c]p value when the comparisons are between the before treatment and after treatment with statin values (Wilcoxon test).

LDL-cholesterol, and AST levels between patients with dyslipidemia before treatment with statins and control subjects and in BMI, serum fasting glucose, total cholesterol, HDL-cholesterol, and AST levels between patients with dyslipidemia after treatment with statins and control subjects.

Table 2 shows median (min–max) values for MN, NPBs, NBUDs, apoptotic and necrotic cell frequencies, frequency of BN cells, NDI, and 8-OHdG levels for each group (control subjects, patients with dyslipidemia before and after treatment with statins). Patients with dyslipidemia before treatment with statins showed higher values in CBMN-cyt assay parameters except NBUD frequency as compared to patients with dyslipidemia after treatment (Table 2). However, these CBMN-cyt assay parameters were significantly reduced to values similar to those of control subjects in patients with dyslipidemia after treatment with statins. Also, frequencies of MN, apoptotic cell and BN cell, NDI, and 8-OHdG levels in patients with dyslipidemia before treatment with statins were found to be significantly higher than in control subjects. No significant differences were found for CBMN-cyt assay parameters except NPB frequency and 8-OHdG levels between patients with dyslipidemia after treatment with statins and control subjects. But, patients with dyslipidemia after treatment with statins showed lower NPB frequency as compared to control subjects.

Also, the associations of age and basic characteristics (BMI, WHR, fasting glucose, triglycerides, total cholesterol, LDL-cholesterol, HDL-cholesterol, AST, ALT) with CBMN-cyt assay parameters and plasma 8-OHdG levels in patients with dyslipidemia before and after treatment with statins and control subjects are evaluated. No correlation was found between duration of treatment and CBMN-cyt assay parameters and plasma 8-OHdG levels in patients with dyslipidemia before and after treatment with statins ($p > 0.05$).

In patients with dyslipidemia before treatment with statins, there were significant inverse associations between NPB frequency and WHR ($r = -0.551$; $p < 0.01$), between frequency of apoptotic cells and ALT ($r = -0.519$; $p < 0.01$), and significant positive associations between NPB frequency and total cholesterol ($r = 0.621$; $p < 0.01$) and LDL-cholesterol ($r = 0.515$; $p < 0.01$).

In patients with dyslipidemia after treatment with statins, there were significant inverse associations between NDI and BMI ($r = -0.410$; $p < 0.05$), between frequency of necrotic cells and triglycerides ($r = -0.450$; $p < 0.05$), between NBUD frequency and AST ($r = -0.425$; $p < 0.05$), and significant positive associations between NBUD frequency and fasting glucose ($r = 0.418$; $p < 0.05$), between frequency of apoptotic cells and HDL-cholesterol ($r = 0.472$; $p < 0.01$), and between NDI and HDL-cholesterol ($r = 0.384$; $p < 0.05$).

In control subjects, there were significant inverse associations between MN frequency and AST ($r = -0.344$; $p < 0.01$), between NPB frequency and WHR ($r = -0.236$; $p < 0.05$), between frequency of apoptotic cells and AST ($r = -0.279$; $p < 0.05$) and ALT ($r = -0.244$; $p < 0.05$), significant positive associations between MN frequency and BMI ($r = 0.281$; $p < 0.05$), and fasting glucose ($r = 0.304$; $p < 0.01$), and total cholesterol ($r = 0.358$; $p < 0.01$), and LDL-cholesterol ($r = 0.318$; $p < 0.01$), between NBUD frequency and fasting glucose ($r = 0.267$; $p < 0.05$), between plasma 8-OHdG levels and age ($r = 0.248$; $p < 0.05$), and BMI ($r = 0.354$; $p < 0.01$), and fasting glucose ($r = 0.475$; $p < 0.01$), total cholesterol ($r = 0.344$; $p < 0.01$), and LDL-cholesterol ($r = 0.313$; $p < 0.01$).

Discussion

Dyslipidemia is a disorder of lipoprotein metabolism and may be manifested by elevated levels of the total cholesterol, LDL-cholesterol, and triglycerides or a decrease in the HDL-cholesterol level in the blood.[1] The prevalence of dyslipidemia has increased dramatically in most developed countries and in many developing countries in recent decades.[24,25] Turkey is a developing country; the prevalence of dyslipidemia is 78.7% in men and 80.4% in women.[25] Low and moderate doses of statin therapy in addition to lifestyle modifications are suggested for primary prevention of

Table 2. CBMN-cyt assay parameters and plasma 8-OHdG levels of patients with dyslipidemia before and after treatment with statins and of control subjects. Data are given as median (min–max).

	Control subjects (n = 20)	Before treatment with statins (n = 30)	After treatment with statins (n = 30)	P value[a]	P value[b]	P value[c]
Frequency of MN (%)	0.75 (0.10–1.60)	1.70 (0.70–7.50)	0.75 (0.10–2.80)	<0.001	0.612	<0.001
Frequency of NPBs (%)	0.50 (0.10–1.30)	0.45 (0.00–3.10)	0.30 (0.00–1.00	0.329	<0.05	<0.05
Frequency of NBUDs (%)	0.25 (0.10–0.50)	0.20 (0.00–1.00)	0.20 (0.00–0.90)	0.919	0.410	0.147
Frequency of apoptotic cells (%)	0.80 (0.10–1.80)	1.05 (0.40–2.40)	0.90 (0.30–2.00)	<0.05	0.296	<0.001
Frequency of necrotic cells (%)	1.35 (0.10–1.90)	1.40 (0.20–2.60	1.00 (0.20–2.30)	0.538	0.180	<0.001
Frequency of BN cells (%)	29.90 (10.60–50.10)	41.80 (11.6–120.20)	28.45 (9.60–78.00)	<0.05	0.890	<0.01
NDI	1.26 (1.11–1.44)	1.35 (1.11–1.85)	1.24 (1.09–1.46)	<0.05	0.781	<0.01
Plasma 8-OHdG levels (ng/mL)	0.69 (0.44–1.02)	1.34 (0.33–1.99)	1.18 (0.56–2.25)	<0.01	<0.001	0.957

BN cells: binucleated cells; MN: micronucleus; NBUDs: nuclear buds; NDI: nuclear division index; NPBs: nucleoplasmic bridges; 8-OHdG: 8-hydroxy-2′-deoxyguanosine.
NDI = [M1 + 2(M2) + 3(M3) + 4(M4)]/N, where M1–M4 represents the total number of lymphocytes with one to four nuclei scored on 1000 viable cells (excluding necrotic and apoptotic cells; M: the number of nuclei; N: the total number of viable cells scored).
[a]p value when the comparisons are between the control and before treatment with statin values (Mann–Whitney U test).
[b]p value when the comparisons are between the control and after treatment with statin values (Mann–Whitney U test).
[c]p value when the comparisons are between the before treatment and after treatment with statin values (Wilcoxon test).

patients with dyslipidemia and also for reducing ASCVD risk.[1,3,4,21]

In the present study, dyslipidemic patients with no current endocrine disease (thyroid disease and diabetes mellitus), hypertension, or cardiovascular disease were treated with the rosuvastatin or atorvastatin worked in the same way for between 3 and 10 months. As expected, treatment with statins decreased serum levels of triglycerides, total cholesterol, and LDL-cholesterol and increased serum levels of HDL-cholesterol in patients with dyslipidemia. The treatment with statins produced no effect on serum fasting glucose, AST, and ALT levels.

In this study, CBMN-cyt assay parameters in peripheral blood lymphocytes and 8-OHdG levels in plasma samples of patients with dyslipidemia before and after treatment with statins and of control subjects were evaluated. The frequencies of MN, apoptotic cell and BN cell, NDI values, and 8-OHdG levels of patients with dyslipidemia before treatment with statins were found to be higher than control subjects. The observed chromosomal DNA damage (MN frequency) in patients with dyslipidemia in the present study is consistent with increased MN frequency in circulating lymphocytes of patients with dyslipidemia and coronary artery disease (CAD).[26,27]

Our data also demonstrate that the frequencies of MN and NPBs as biomarkers of chromosomal DNA damage are reduced in patients with dyslipidemia after treatment compared with before treatment with statins. Even, the frequency of NPBs in patients with dyslipidemia was reduced below that of control subjects. The total cholesterol and LDL-cholesterol levels showed positive associations with the frequency of NPBs in patients with dyslipidemia prior to treatment with statins. Hence higher lipid levels may contribute to the observed chromosomal DNA damage in these patients.

To our knowledge, this is the first study to demonstrate that statin therapy is associated with decreased CBMN-cyt assay parameters in dyslipidemic patients. Only one *in vivo* study has shown that there is no significant increase in the number of micronucleated polychromatic erythrocytes in bone marrow of mice after single oral doses of atorvastatin were administered.[28] However, it is possible to find some *in vitro* CBMN assay studies about human lymphocytes and statins. But the results of these studies are contradictory; it was found that atorvastatin significantly decreased MN frequency induced by ionizing radiation in human lymphocytes,[29] while rosuvastatin significantly increased MN frequency at all concentrations in human lymphocytes.[30]

Dyslipidemia can be associated with oxidative stress and oxidative DNA damage. Manfredini *et al.*[12] showed that dyslipidemic T2D patients not treated with statin therapy have higher oxidative DNA damage indices than non-dyslipidemic T2D patients, dyslipidemic T2D patients receiving statin treatment, and healthy age-matched control subjects by comet assay. Several clinical studies suggest that simvastatin and atorvastatin therapy significantly reduces oxidative DNA damage in hyperlipidemic patients when using the comet assay.[13,14] Inoue *et al.*[31] demonstrated that the 8-OHdG levels in dyslipidemic patients decrease after fluvastatin therapy, which is in contrast to our data because no significant differences were observed for 8-OHdG levels between the patients with dyslipidemia before or after treatment with a statin. Taken together, from our results, it might be suggested that the reduced frequencies of MN and NPBs in statin-treated dyslipidemic patients did not depend on the oxidative damage at the DNA level but rather on the serum triglycerides, total cholesterol, and LDL-cholesterol levels.

The present study has shown significant increases in the frequency of apoptotic cells as a biomarker of cytotoxicity and in BN cell frequency and NDI values as biomarkers of cytostasis in patients with dyslipidemia prior to treatment with statins compared to those in control subjects. However, in contrast, Bhat *et al.*[27] had observed lower NDI values in CAD patients than in controls. After statin treatment we also found that the frequencies of apoptotic cells, BN cells, and NDI values are decreased in patients with dyslipidemia. Similarly, an *in vitro* study had shown that rosuvastatin decreased the cytokinesis-block proliferation indices in human peripheral blood lymphocytes, but this effect was not statistically significant.[30]

Additionally, experimental and clinical data suggest that statins may reduce the risk of some cancers, such as those of the prostate, colon, pancreas, and breast.[32–35] The possible antineoplastic mechanisms of statins are an area of active investigation. Interestingly, in new therapeutic approaches of pancreatic ductal adenocarcinoma (PDA), statins have been reported to promote p21 expression, Rb tumor suppressor protein dephosphorylation, and repression of E2F target genes associated with proliferation in primary patient derived tumor cells and established PDA lines.[36] The CBMN-cyt assay used in this is a comprehensive method used for measuring chromosomal DNA damage (frequencies of MN, NPBs, and NBUDs), cell proliferation (cytostasis, NDI), and cell death (cytotoxicity, frequencies of apoptotic and necrotic cell) in variety of human cells.[7] This method is also used as biomarker in cancer risk assessment.[37–39] Thus, our findings may be confirmed by the association between statin use and reduced risk of cancer, but how blood cholesterol levels might affect risk of some cancers and statins might decrease cancer risk in humans is still unclear.

The most obvious limitation of this study is the small number of patients with dyslipidemia enrolled. In the initiation of study, the number of patients is 71. But the number of patients who completed the treatment is 30. Thus, the study could be performed on 30 patients due to stop of taking statin and lack of follow-up after treatment..

Conclusion

In the present study, statin therapy decreased chromosomal DNA damage (MN and NPB frequencies) and NDI values in patients with dyslipidemia by possible molecular reasons independent of oxidative DNA damage. In addition to the LDL-cholesterol-lowering and known pleiotropic effects of the statins, the findings of this study suggest that statins may have a genome protective and beneficial effect on cytotoxicity and cytostasis in the lymphocytes of patients with dyslipidemia.

Authors' contributions: HD-A, FB, and ANC-D contributed to the conception and design. HD-A, ANC-D, FB, and OB performed the experiments and contributed to the collection of patients. HD-A, FB, DK, and PPT drafted the article and critically revised the article. All authors approved the final version.

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REFERENCES

1. Catapano AL, Graham I, De Backer G, Wiklund O, Chapman MJ, Drexel H, Hoes AW, Jennings CS, Landmesser U, Pedersen TR, Reiner Z, Riccardi G, Taskinen MR, Tokgozoglu L, Verschuren WM, Vlachopoulos C, Wood DA, Zamorano JL. 2016 ESC/EAS guidelines for the management of dyslipidaemias: the task force for the management of dyslipidaemias of the European society of cardiology (ESC) and European atherosclerosis society (EAS) developed with the special contribution of the European Association for cardiovascular prevention & rehabilitation (EACPR). *Atherosclerosis* 2016;**253**:281–344.
2. Akhabue E, Rittner SS, Carroll JE, Crawford PM, Dant L, Laws R, Leo MC, Puro J, Persell SD. Implications of American College of Cardiology/American Heart Association (ACC/AHA) cholesterol guidelines on statin underutilization for prevention of cardiovascular disease in diabetes mellitus among several US networks of community health centers. *J Am Heart Assoc* 2017;**6**:e005627
3. Graham IM, Catapano AL, Wong ND. Current guidelines on prevention with a focus on dyslipidemias. *Cardiovasc Diagn Ther* 2017;**7**:S4–S10
4. Jacobson TA, Ito MK, Maki KC, Orringer CE, Bays HE, Jones PH, McKenney JM, Grundy SM, Gill EA, Wild RA, Wilson DP, Brown WV. National lipid association recommendations for patient-centered management of dyslipidemia: part 1-executive summary. *J Clin Lipidol* 2014;**8**:473–88
5. Fenech M. The in vitro micronucleus technique. *Mutat Res-Fund Mol M* 2000;**455**:81–95
6. Fenech M. Cytokinesis-block micronucleus assay evolves into a "cytome" assay of chromosomal instability, mitotic dysfunction and cell death. *Mutat Res-Fund Mol M* 2006;**600**:58–66
7. Fenech M. Cytokinesis-block micronucleus cytome assay. *Nat Protoc* 2007;**2**:1084–104
8. Fenech M, Kirsch-Volders M, Natarajan AT, Surralles J, Crott JW, Parry J, Norppa H, Eastmond DA, Tucker JD and Thomas P. Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells. *Mutagenesis* 2011;**26**:125–32
9. Pilger A, Rudiger HW. 8-Hydroxy-2′-deoxyguanosine as a marker of oxidative DNA damage related to occupational and environmental exposures. *Int Arch Occup Environ Health* 2006;**80**:1–15
10. Harangi M, Remenyik E, Seres I, Varga Z, Katona E, Paragh G. Determination of DNA damage induced by oxidative stress in hyperlipidemic patients. *Mutat Res-Gen Tox En* 2002;**513**:17–25
11. Pereira RD, Tatsch E, Bochi GV, Kober H, Duarte T, Montagner G, da Silva JEP, Duarte M, da Cruz IBM, Moresco RN. Assessment of oxidative, inflammatory, and fibrinolytic biomarkers and DNA strand breakage in hypercholesterolemia. *Inammation* 2013;**36**:869–77
12. Manfredini V, Biancini GB, Vanzin CS, Dal Vesco AMR, Cipriani F, Biasi L, Tremea R, Deon M, Peralba MDR, Wajner M, Vargas CR. Simvastatin treatment prevents oxidative damage to DNA in whole blood leukocytes of dyslipidemic type 2 diabetic patients. *Cell Biochem Funct* 2010;**28**:360–6
13. Shin MJ, Cho EY, Yang Y, Lee JH, Shim WH, Cho SY, Rim SJ, Kang SM, Ha JW, Ko YG, Kim SS, Park HY, Chung N. A beneficial effect of simvastatin on DNA damage in 242T allele of the NADPH oxidase p22phox in hypercholesterolemic patients. *Clin Chim Acta* 2005;**360**:46–51
14. Harangi M, Seres I, Varga Z, Emri G, Szilvassy Z, Paragh G, Remenyik E. Atorvastatin effect on high-density lipoprotein-associated paraoxonase activity and oxidative DNA damage. *Eur J Clin Pharmacol* 2004;**60**:685–91
15. Donmez-Altuntas H, Bayram F, Bitgen N, Ata S, Hamurcu Z, Baskol G. Increased chromosomal and oxidative DNA damage in patients with multinodular goiter and their association with cancer. *Int J Endocrinol* 2017;**2017**:2907281
16. Donmez-Altuntas H, Sahin F, Bayram F, Bitgen N, Mert M, Guclu K, Hamurcu Z, Aribas S, Gundogan K, Diri H. Evaluation of chromosomal damage, cytostasis, cytotoxicity, oxidative DNA damage and their association with body-mass index in obese subjects. *Mutat Res-Gen Tox En* 2014;**771**:30–6
17. Bitgen N, Donmez-Altuntas H, Bayram F, Cakir I, Hamurcu Z, Diri H, Baskol G, Senol S, Durak AC. Increased micronucleus, nucleoplasmic bridge, nuclear bud frequency and oxidative DNA damage associated with prolactin levels and pituitary adenoma diameters in patients with prolactinoma. *Biotech Histochem* 2016;**91**:128–36
18. Bayram F, Bitgen N, Donmez-Altuntas H, Cakir I, Hamurcu Z, Sahin F, Simsek Y, Baskol G. Increased genome instability and oxidative DNA damage and their association with IGF-1 levels in patients with active acromegaly. *Growth Horm IGF Res* 2014;**24**:29–34
19. Hamurcu Z, Cakir I, Donmez-Altuntas H, Bitgen N, Karaca Z, Elbuken G, Bayram F. Micronucleus evaluation in mitogen-stimulated lymphocytes of patients with acromegaly. *Metabolism* 2011;**60**:1620–6
20. Hamurcu Z, Bayram F, Kahriman G, Donmez-Altuntas H, Baskol G. Micronucleus frequency in lymphocytes and 8-hydroxydeoxyguanosine level in plasma of women with polycystic ovary syndrome. *Gynecol Endocrinol* 2010;**26**:590–5
21. Stone NJ, Robinson JG, Lichtenstein AH, Merz CNB, Blum CB, Eckel RH, Goldberg AC, Gordon D, Levy D, Lloyd-Jones DM, McBride P, Schwartz JS, Shero ST, Smith SC, Watson K, Wilson P. 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults a report of the American college of cardiology/American heart association task force on practice guidelines. *J Am Coll Cardiol* 2014;**63**:2889–934
22. Gundogan K, Donmez-Altuntas H, Hamurcu Z, Akbudak IH, Sungur M, Bitgen N, Baskol G, Bayram F. Evaluation of chromosomal DNA damage, cytotoxicity, cytostasis, oxidative DNA damage and their relationship with endocrine hormones in patients with acute organophosphate poisoning. *Mutat Res-Gen Tox En* 2018;**825**:1-7
23. Eastmond DA, Tucker JD. Identification of aneuploidy-inducing agents using cytokinesis-blocked human-lymphocytes and an antikinetochore antibody. *Environ Mol Mutagen* 1989;**13**:34–43
24. Sun GZ, Li Z, Guo L, Zhou Y, Yang HM, Sun YX. High prevalence of dyslipidemia and associated risk factors among rural Chinese adults. *Lipids Health Dis* 2014;**13**:189
25. Bayram F, Kocer D, Gundogan K, Kaya A, Demir O, Coskun R, Sabuncu T, Karaman A, Cesur M, Rizzo M, Toth PP, Gedik V. Prevalence of dyslipidemia and associated risk factors in Turkish adults. *J Clin Lipidol* 2014;**8**:206–16
26. Andreassi MG, Botto N, Simi S, Casella M, Manfredi S, Lucarelli M, Venneri L, Biagini A, Picano E. Diabetes and chronic nitrate therapy as co-determinants of somatic DNA damage in patients with coronary artery disease. *J Mol Med* 2005;**83**:279–86

27. Bhat MA, Mahajan N, Gandhi G. DNA and chromosomal damage in coronary artery disease patients. *Excli J* 2013;**12**:872–84
28. Ciaravino V, Kropko ML, Rothwell CE, Hovey CA, Theiss JC. The genotoxicity profile of atorvastatin, a new drug in the treatment of hypercholesterolemia. *Mutat Res-Gen Tox En* 1995;**343**:95–107
29. Hosseinimehr SJ, Izakmehri M, Ghasemi A. In vitro protective effect of atorvastatin against ionizing radiation induced genotoxicity in human lymphocytes. *Cell Mol Biol* 2015;**61**:68–71
30. Berber AA, Celik M, Aksoy H. Genotoxicity evaluation of HMG CoA reductase inhibitor rosuvastatin. *Drug Chem Toxicol* 2014;**37**:316–21
31. Inoue T, Inoue K, Maeda H, Takayanagi K, Morooka S. Immunological response to oxidized LDL occurs in association with oxidative DNA damage independently of serum LDL concentrations in dyslipidemic patients. *Clin Chim Acta* 2001;**305**:115–21
32. Denoyelle C, Vasse M, Korner M, Mishal Z, Ganne F, Vannier JP, Soria J, Soria C. Cerivastatin, an inhibitor of HMG-CoA reductase, inhibits the signaling pathways involved in the invasiveness and metastatic properties of highly invasive breast cancer cell lines: an in vitro study. *Carcinogenesis* 2001;**22**:1139–48
33. Kamigaki M, Sasaki T, Serikawa M, Inoue M, Kobayashi K, Itsuki H, Minami T, Yukutake M, Okazaki A, Ishigaki T, Ishii Y, Kosaka K, Chayama K. Statins induce apoptosis and inhibit proliferation in cholangiocarcinoma cells. *Int J Oncol* 2011;**39**:561–8
34. Babcook MA, Joshi A, Montellano JA, Shankar E, Gupta S. Statin use in prostate cancer: an update. *Nutr Metab Insights* 2016;**9**:43–50
35. Cardwell CR, Hicks BM, Hughes C, Murray LJ. Statin use after colorectal cancer diagnosis and survival: a population-based cohort study. *J Clin Oncol* 2014;**32**:3177–83
36. Villarino N, Signaevskaia L, van Niekerk J, Medal R, Kim H, Lahmy R, Scully K, Pinkerton A, Kim S, Lowy A, Itkin-Ansari P. A screen for inducers of bHLH activity identifies pitavastatin as a regulator of p21, Rb phosphorylation and E2F target gene expression in pancreatic cancer. *Oncotarget* 2017;**8**:53154–67
37. El-Zein RA, Lopez MS, D'Amelio AM, Liu M, Munden RF, Christiani D, Su L, Tejera-Alveraz P, Zhai R, Spitz MR and Etzel CJ. The cytokinesis-blocked micronucleus assay as a strong predictor of lung cancer: extension of a lung cancer risk prediction model. *Cancer Epidem Biomar* 2014;**23**:2462–70
38. Bonassi S, El-Zein R, Bolognesi C, Fenech M. Micronuclei frequency in peripheral blood lymphocytes and cancer risk: evidence from human studies. *Mutagenesis* 2011;**26**:93–100
39. Bonassi S, Znaor A, Ceppi M, Lando C, Chang WP, Holland N, Kirsch-Volders M, Zeiger E, Ban S, Barale R, Bigatti MP, Bolognesi C, Cebulska-Wasilewska A, Fabianova E, Fucic A, Hagmar L, Joksic G, Martelli A, Migliore L, Mirkova E, Scarfi MR, Zijno A, Norppa H, Fenech M. An increased micronucleus frequency in peripheral blood lymphocytes predicts the risk of cancer in humans. *Carcinogenesis* 2007;**28**:625–31

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