Research Article (Araştırma Makalesi)

Investigation of Genotoxic Damage in Overweight Individuals Using the Comet Assay

Aşırı Kilolu Kişilerde Olası Genotoksik Hasarın Comet Deneyi ile Analizi

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Özet

Amaç: Son yıllarda yapılmış olan araştırmalar ile obezitenin, DNA zincir kırıklarının onarım mekanizmasını değiştirdiği ortaya çıkarılmıştır. Ayrıca beden kitle indeksinde artış ile genomik kararsızlık arasında ilişki tespit edilmiştir. Sunulan çalışmada aşırı kilolu bireylerin muhtemel genotoksik hasarının araştırılması amaçlanmış olup periferal kan örneklerinde Tek Hücre Jel Elektroforezi deneyi kullanılarak olası genotoksik hasar düzeyi hesaplanmıştır.

Gereç ve Yöntemler: Sunulan çalışmada 18 yaşından büyük aşırı kilolu ya da normal kiloya sahip bireylerin periferal lenfositlerinde olası DNA hasarı Comet Deneyi ile analiz edilmiştir. Sonuçlar SPSS analiz programı kullanılarak istatistiksel olarak karşılaştırılmıştır.

Bulgular: Sunulan araştırmaya yaş ortalaması 30.13±7.97 olan 23 kadın ve yaş ortalaması 38.13±10.63 olan 32 erkek (21 normal kilolu ve 34 aşırı kilolu) toplamda 55 gönüllü katılmıştır. DNA hasarının göstergesi olan kuyruk momenti değeri tüm bireylerde ortalama 1.21±0.45'dir. Aşırı kilolu kişilerin kuyruk momenti değeri ortalama 1.29±0.46 olarak bulunmuştur. Bu değer normal kiloya sahip bireylerin kuyruk momenti sonuçları (1.09±0.40) ile karşılaştırıldığında istatistiksel olarak anlamlı derecede fark bulunmamıştır ($p>0.05$). Çalışma sonuçlarımıza göre beden kitle indeksinde artış ile DNA hasarı arasında anlamlı fark bulunmamıştır ($p>0.05$).

Sonuç: Sunulan çalışma Türkiye'deki yetişkin bireylerde aşırı kiloluluk ve DNA hasar düzeyinin değerlendirildiği ilk çalışma olma özelliğindedir. Sunulan çalışmada Comet Deneyi bulgularına göre beden kitle indeksi ve genotoksik hasar arasında ilişki bulunmadığı tespit edilmiş olup gelecekte DNA hasar düzeyinin farklı genotoksisite testleriyle araştırılacağı yeni çalışmaların yapılması önerilmektedir.

Anahtar kelimeler: Comet deneyi, DNA hasarı, Genotoksisite, Obezite, Tek hücre jel elektroforezi

Abstract

Objective: Research has revealed that obesity changed the repair mechanism of DNA chain breaks. Also, the increase in body mass index is found out to be associated with genomic instability. In this study, it was aimed to investigate the possible genotoxic damage of overweight individuals.

Material and Methods: In the present study the level of genotoxic damage was calculated in Turkish adult peripheral blood samples using Single Cell Gel Electrophoresis assay. The results of possible DNA damage levels belonging to overweight people were compared statistically by SPSS analysis program with the results of normal-weight people.

Results: Fifty five volunteers (21 normal weight and 34 overweight); 23 women, mean age=30.13±7.97 and 32 men, mean age=38.13±10.63 participated in the study. Tail moment is an average of 1.21±0.45 in all individuals. Tail moment value was found of overweight people as 1.29±0.46. When this value was compared with the results of individuals with normal weight (1.09±0.40), statistically no significant difference was determined ($p>0.05$). According to results, no significant difference was found between the increase in body mass index and DNA damage ($p>0.05$).

Conclusion: The present study is the first study which gives information about the level of DNA damage relation with being overweight in adults of Turkey. In the presented study, it was determined that there was no relationship between body mass index and genotoxic damage according to the findings of the comet assay, and we recommended that new studies should be conducted in the future to investigate the level of DNA damage with different genotoxicity tests.

Keywords: Comet assay, DNA damage, Genotoxicity, Obesity, Single cell gel electrophoresis

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INTRODUCTION

Obesity, affects the whole world which is widespread not only in adults but also in children. Obesity ratio has tripled in the last fifty years (1). Recent studies presented to the scientific literature in years have shown that obesity may affect genome stability. The relationship between increased body mass index (BMI) level and DNA damage is a fairly new research topic. They reported that accumulation of DNA damage and development of diseases associated with BMI increase (2,3). It is known that the increase in the BMI level changes the repair mechanism of DNA double strand breaks caused by genotoxic chemicals (4). Scientific studies revealed that individuals with high BMI levels have twice as many DNA double-strand breaks, single-strand breaks, oxidized bases, and DNA damage compared to individuals of normal weight (5,6). Increased BMI can lead to increase in DNA damage or impairment in DNA repair, and as a result, the accumulation of DNA damage in the cell can cause inflammation, changes in gene expression and disorders in cellular metabolism (4). Animal experiments results indicate that there is an increase in mitochondrial DNA damage in animals fed a high fat diet (7,8). Results of studies conducted on young adults aged 18-30 years showed a negative correlation between BMI and nucleotide excision repair capacity (9). According to the results of the study comparing the DNA damage of peripheral lymphocytes belonging to obese and normal weight adolescents it has been shown that the increase in BMI can change the mechanism of DNA double strand breakage repair caused by genotoxic agents (10). In addition, weight gain is recognized as an important risk factor for the development of cancer. Overweight and obesity in adults associated with esophagus, kidney, pancreas, gastric cardia, multiple myeloma, colon, rectum, postmenopausal breast, endometrium, ovary and gall bladder cancer types. Overweight or obese people constitute 20% of all cancer cases (11). Meta-analysis study results revealed that there is a relationship between weight gain and cancer (12,13). Studies over the past few years have shown that an increase in BMI can affect genome stability. In the presented study, possible effects of overweight on genotoxic damage were investigated.

MATERIALS AND METHODS

Study Group

A total of 55 volunteers, aged ≥18 were included in the present study. To evaluate the genotoxic damage in present study, blood samples were taken from each individual into a heparin tube. The permission of the Süleyman Demirel University Faculty of Medicine clinical research ethics committee (dated 17.11.2020 and numbered 2020/367) has been obtained. This study have been conducted in accordance with the Helsinki Declaration of Principles. A consent form was signed by each individual participating in the study. BMI values of volunteers were calculated according to World Health Organisation-WHO (1) criteria by dividing the weight in kilograms by the square of the height in meters, and the individuals were classified as normal and overweight. Thirty-four overweight volunteers were included in the study. The Comet Assay results of individuals with overweight were statistically compared with the results of twenty-one healthy individuals, who were not exposed to smoking and who had normal weight as a result of BMI calculation.

Single Cell Gel Electrophoresis (The Comet Assay)

In the presented study, possible DNA damage level was analysed by Comet Assay. Comet Assay was applied according to the Tice et al. (14) method. For the detection of possible DNA damage, the values of DNA tail length (μm), tail density (%DNA), and tail moment parameters were recorded using Comet Experiment III Imaging Analysis System (Perceptive Instruments, Steeple Bumpstead, UK) and statistically analysed. All preparations were coded to reduce reader errors and were evaluated in a single blind.

Statistical Analysis

The demographic characteristics of the volunteers participating in the study were presented by calculating the mean and standard deviation (Std) values. In the power analysis performed according to the BMI mean and Std values of study groups. 99% power analysis value was obtained at α=0.05 significance level. According to the result of this power analysis, it is seen that the sample size is sufficient. The data obtained with the result of Comet Assay were analysed by using Statistical Package for Social Sciences (SPSS). If the p value result is found to be less than 0.05, it is considered to be statistically significant. While making comparisons between groups, the distribution of variables such as age, length, weight and BMI were taken into account and the data were evaluated using the Survival Curve.

RESULTS

In the present study, 21 normal weight and 34 overweight volunteers were participated. The mean BMI of normal weight women participating in the study was 22.47±2.04, and the mean BMI of normal weight men was 22.63±2.31 kg/m². While the average BMI of overweight women was determined as 27.11±1.25, the average BMI of overweight men was found to be 26.98±1.49 kg/m². When the demographic characteristics of the study group were evaluated between normal weight and overweight individuals, a statistically significant difference was found in age, weight, height, BMI. The demographic characteristics of the study group was presented in **Table 1**.

In the present study, the level of genetic damage in peripheral lymphocytes in normal weight and overweight individuals was analysed using the Comet Assay. Mean values and standard deviations of tail length (μm), tail moment, tail intensity (%) results, which are the parameters of Comet Assay, were presented in **Table 2**.

According to the results of the study, the statistical significance level between normal weight and overweight individuals was determined respectively for tail length, tail moment and tail density, which are Comet experiment parameters. The statistical significance level between normal weight and overweight individuals for tail length, tail moment and tail density, was found p=0.717; p=0.106; p=0.107 respectively. In the present study we indicate that there was no significant difference between the increase in body mass index and DNA damage (p>0.05). **Table 3** was shown Comet Assay parameters of normal and overweight individuals.

Survival curve results of Comet Assay parameters of tail moment, tail lenght and, tail intensity was shown **Figure 1**, **Figure 2**, and **Figure 3** respectively. Also, Comet Assay image of normal DNA and damaged DNA was given **Figure 4** and **Figure 5**.

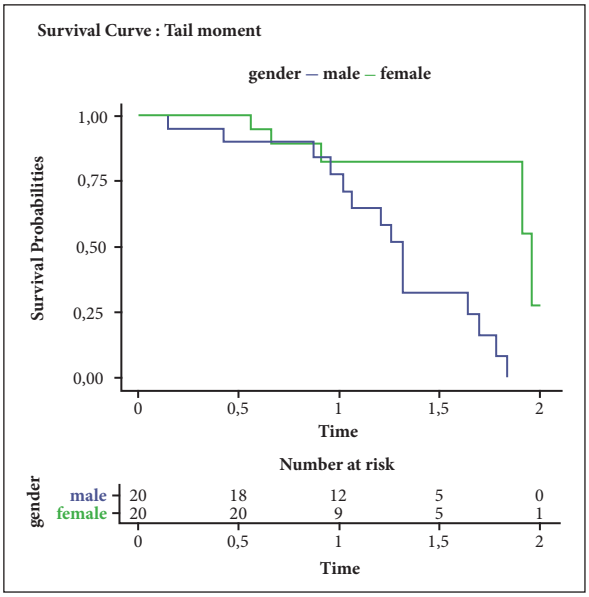

Figure 1. Survival Curve of Tail Moment

Table 1. Demographic data of study groups

Parameters	Normal Weight (n=21)		Overweight (n=34)		*p* value
	Female (n=16)	Male (n=5)	Female (n=7)	Male (n=27)	
Age (Year)	28.19±4.79	33.40±10.57	34.57±11.94	39.04±10.61	0.001
Height (cm)	164.44±5.51	173.60±4.16	163.00±9.90	177.26±7.24	0.002
Weight (kg)	60.79±6.92	68.20±7.19	72.00±6.58	84.96±8.77	<0.001
BMI (kg/m²)	22.47±2.04	22.63±2.31	27.11±1.25	26.98±1.49	<0.001

BMI: Body mass index

Table 2. Comet Assay results in females and males

Comet parameters	Normal Weight		Overweight	
	Female (n=16)	Male (n=5)	Female (n=7)	Male (n=27)
Tail length	28.45±6.03	30.94±8.20	31.04±4.96	29.18±3.61
Tail moment	1.13±0.42	0.97±0.35	1.22±0.56	1.31±0.44
Tail intensity	5.29±2.19	4.49±1.95	5.82±2.79	6.04±1.64

Table 3. Comet Assay results in normal and overweight study groups

Comet parameters	Normal Weight (n=21)	Overweight (n=34)	*p* values
Tail length	28.98±6.37	29.56±3.91	0.717
Tail moment	1.09±0.40	1.29±0.46	0.106
Tail intensity	5.10±2.11	6.00±1.89	0.107

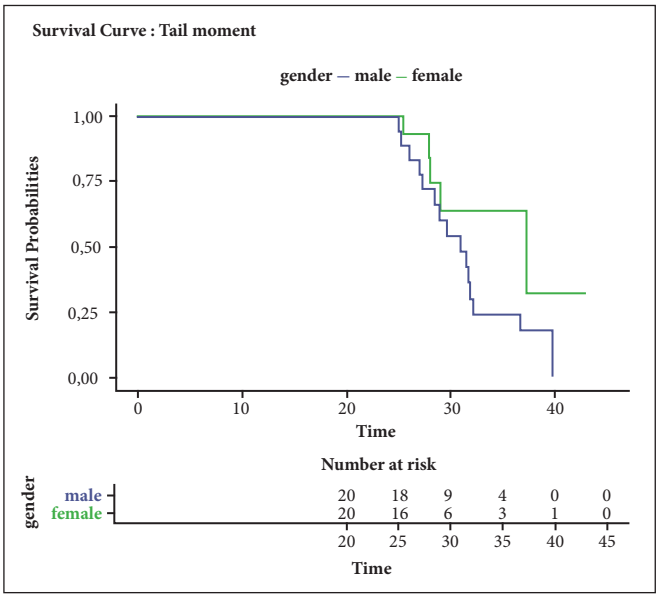

Figure 2. Survival Curve of Tail Lenght

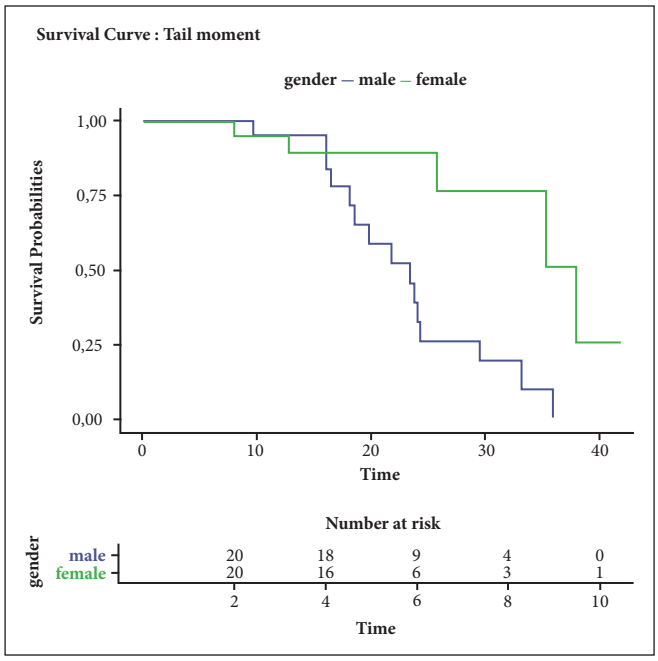

Figure 3. Survival Curve of Tail Intensity

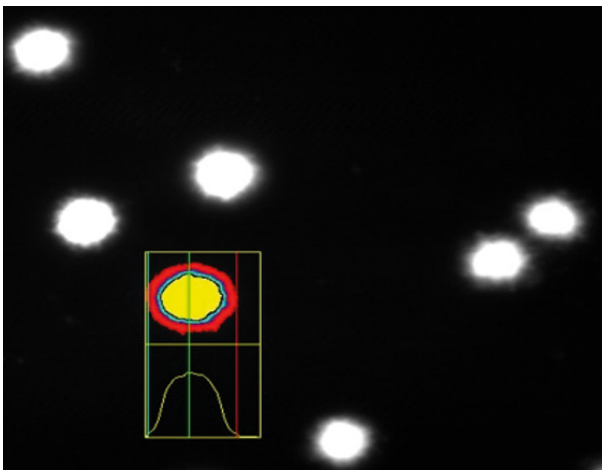

Figure 4. Comet Image of Normal DNA

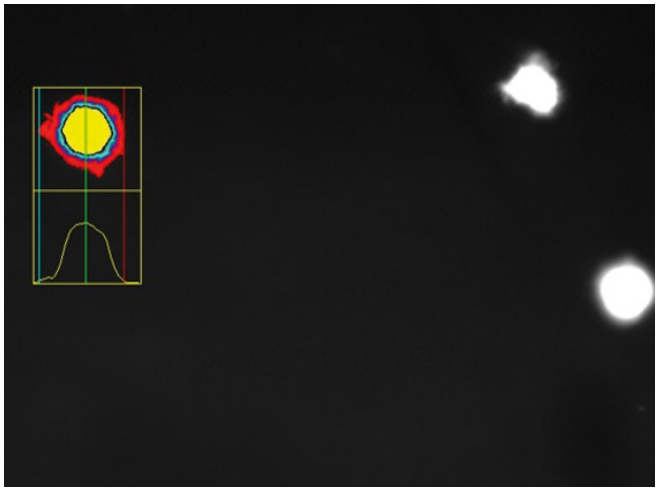

Figure 5. Comet Image of Damaged DNA

DISCUSSION

In the last decade, a number of studies have been conducted to examine the relationship between obesity and DNA damage (13-17). Twenty four studies on somatic cells or sperm cells have shown a positive relationship between increased BMI and DNA damage, while 22 studies have shown no correlation or negative relationship (16). Genomic damage levels measures micronuclei frequencies or chromosomal aberrations in human lymphocytes. A significant correlation was found between micronucleus frequencies, and body mass index (18). Also, studies with overweight/obese participants showed a positive association with chromosomal damage. Obese subjects demonstrated significantly higher frequencies of DNA damage, than normal weight and overweight subjects (19). Study results indicate that obesity associated with interstitial deletion of chromosomes and obesity-associated gene expression and weight gain (20). A statistically significant difference in DNA damage in overweight or obese children compared to the control group was revealed by the Comet Assay, which is an important genotoxicity test (21). The results of the study on human lymphocytes compared to normal-weight individuals showed greater DNA damage in obese subjects (22).

In scientific literature a numerous studies which DNA damage in obese individuals are compared with normal weight individuals using genotoxicity tests and where there is a statistically significant difference find (2,21-23). Włodarczyk et al. (2) a study conducted on 114 people, it was revealed that DNA damage in obese individuals was twice as high ($p<0.001$) compared to

normal weight individuals. Gandhi and Kaur analysed DNA damage in obese individuals with the Comet Assay, which is an important genotoxicity test that has been widely used in recent years. As a result of the experiment in obese individuals, they revealed the frequency of DNA damage as 77.77±1.12, the damage index as 47.34±0.79 and the amount of DNA migration as 29.14±0.93 μm. A statistically significant difference ($p<0.001$) in DNA damage was shown compared with the control group (15).

Dönmez-Altuntaş et al. (24) concluded that a positive correlation between increased body mass index (BMI) and DNA damage. Another study supporting Cerdá et al. (25), they found a positive correlation between BMI and DNA damage. Zaki et al. (17) evaluate obesity and DNA damage using the Comet Assay in 172 women, they found that obese women have higher DNA damage. Although there are many studies in the literature that show a positive relationship between obesity and DNA damage according to the study of Setayesh et al. (16) and Milić et al. (26) there is a weak relationship between increase in body weight and DNA damage.

Epidemiological studies conducted in recent years put forward that the increase in BMI may have effects on genome stability. It is known that DNA damage also plays a role in the etiology of many diseases, especially cancer. Because of ability to trigger cancer formation, should be fought with weight gain. In the present study, possible genotoxic effects in overweight individuals analysed with the Comet Assay. Our study results show that there is no statistically significant relationship between overweight and DNA damage.

It is thought that our results will form a step for new studies to be conducted on more people and using more than one genotoxicity test, due to the nature of science.

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Conflict of Interest and Financial Status: The authors declare that they have no competing interest.

Ethical Approval: The study was approved by the Ethic Committee of Suleyman Demirel University Faculty of Medicine clinical research ethics committee (2020/367). This study have been conducted in accordance with the Helsinki Declaration of Principles.

Research Contribution Rate Statement Summary: The authors declare that, they have contributed equally to the manuscript.

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