ORIGINAL ARTICLE

AHI EVRAN MEDICAL JOURNAL
E-ISSN: 2619 - 9203

Assessment of cfDNA Levels in Saliva Samples of Stressed Young Adults: Preliminary Study

Stres Altındaki Genç Yetişkinlerin Tükürük Örneklerinde cfDNA Düzeylerinin Değerlendirilmesi: Ön Çalışma

Ekin ÇELİK[1] Miraç Berkay KÖKSAL[2] Hacı Mehmet AKBULUT[2] Selahattin Ata AYDIN[2]

Yiğit Emre KURT[2] Can OKAN[2] Simay ÇETİNKAYA[2]

ÖZ

Amaç: Bu pilot çalışmada, ikinci sınıf tıp öğrencilerinin sınav öncesi ve sonrasında tükürük örneklerindeki hücre dışı DNA (hdDNA, cfDNA) düzeylerinin incelenmesi amaçlanmıştır.
Araçlar ve Yöntem: Stres profilleri DASS-21 ölçeği ile değerlendirilen 20 öğrencinin sınav öncesi ve sonrası tükürük örnekleri alınarak hücre dışı DNA izole edilmiştir. İzole edilen DNA'lar spektrofotometre ve otomatik elektroforez sistemi ile ölçülmüştür.
Bulgular: Spektrofotometre analizleri, sınav sonrası öğrencilerden alınan cfDNA miktarlarında anlamlı bir azalma göstermiştir ($p \leq 0.05$). Bu fark otomatik elektroforez sistemi ile doğrulanmış ve özellikle 40-200 bp aralığındaki cfDNA miktarlarının stresin azalmasıyla birlikte azaldığı tespit edilmiştir ($p \leq 0.05$).
Sonuç: Psikososyal stres hdDNA salınımını etkilemektedir. Bu çalışmada, tükürük örneklerinde bulunan hdDNA miktarlarının stresle ilişkili olarak anlamlı değişiklikler gösterdiği raporlanmıştır. Bu ön çalışma, tükürük numunelerindeki hdDNA'nın sağlıklı bireylerde stresin potansiyel biyolojik bir belirteci olarak değerlendirilebileceğini ortaya koymuştur.

Anahtar Kelimeler: biyobelirteç; hdDNA; plazma

ABSTARCT

Purpose: The aim of this pilot study was to examine the levels of cell-free DNA (cfDNA) in the saliva samples of second-year medical students before and after a stress-inducing event, exams.
Materials and Methods: Saliva samples were collected from 20 students, whose stress profiles were assessed using the DASS-21 scale, both before and after the exams. Cell-free DNA was isolated from these samples, and measured using spectrophotometry and automated electrophoresis system.
Results: Spectrophotometric analysis revealed a significant decrease in cfDNA levels in the saliva samples collected after the exams ($p \leq 0.05$). This difference was confirmed by the automated electrophoresis system, particularly showing a reduction in cfDNA amounts in the 40-200 bp range with the reduction of stress ($p \leq 0.05$).
Conclusion: Psychosocial stress affects the release of cell-free DNA. This study reports that the amount of cfDNA found in saliva samples significantly changes in relation to stress. This preliminary study suggests that cfDNA in saliva samples could potentially serve as a biological marker for stress in healthy individuals.

Keywords: biomarker; cfDNA; plasma

Received: 29.07.2024; Accepted: 19.08.2024

[1]Department of Medical Biology, Faculty of Medicine, Kırşehir Ahi Evran University, Kırşehir, Türkiye.
[2]Faculty of Medicine, Kırşehir Ahi Evran University, Kırşehir, Türkiye.
Corresponding Author: Ekin Çelik, Department of Medical Biology, Faculty of Medicine, Kırşehir Ahi Evran University, Kırşehir, Türkiye.
e-mail: ekin.celik@ahievran.edu.tr

How to cite: Çelik E, Köksal MB, Akbulut HM, Aydın SA, Kurt YE, Okan C, Çetinkaya S. Assessment of cfDNA levels in saliva samples of stressed young adults: preliminary study. Ahi Evran Med J. 2025;9(1):12-16. DOI: 10.46332/aemj.1524197

INTRODUCTION

DNA that is present in blood plasma, serum, cerebrospinal fluid, saliva, and urine without being associated with cells is known as cell-free DNA (cfDNA) or extracellular DNA (ecDNA).[1,2] The exact biological sources of cell-free DNA are still not fully understood; it is suggested that they may originate from mechanisms like active secretion, apoptosis, necrosis, or netosis.[3,4]

Elevated amounts of cell-free DNA (cfDNA) in the blood, whether originating from the genome or mitochondria, are distinctive indicators of both acute systemic inflammatory reactions and long-term inflammation.[5] Such heightened levels have been observed following events like trauma, sepsis, stroke, ischemia/reperfusion injury, and myocardial infarction, as well as in individuals with cancer, autoimmune diseases, cardiovascular conditions, and metabolic disorders.[6] In these situations, cfDNA has been firmly established as a dependable and consistent biomarker. Measuring cfDNA levels holds promise as a valuable clinical tool for assessing risk and monitoring therapy effectiveness across various inflammatory contexts.[7]

Stress is a common psychophysiological reaction produced by the body in response to adverse, challenging, and difficult situations or stressors.[8] It impacts the immune system and triggers peripheral inflammatory pathways, resulting in the secretion of certain biomolecules like hormones, and as recently revealed, cfDNA.[9]

The effect of psychosocial stress on plasma cell-free DNA (cfDNA) levels has become a focal point in recent years.[6-12] Czamanski-Cohen et al, studied the relationship between elevated cortisol levels and cfDNA amounts in plasma and showed a positive correlation.[10] Another study indicated that stress reduction might promote changes that result in lowered plasma cfDNA levels.[11] Shan and his colleagues conducted a case-control study in 2024, which showed increased stress levels resulted in elevated cfDNA levels in plasma.[12]

MATERIALS and METHODS

Study Design

This pilot study, conducted at the Kırşehir Ahi Evran University Faculty of Medicine from October 2023 to May 2024, included a sample of 20 participants. The DASS-21 scale, a tool designed to evaluate symptoms of depression, anxiety, and stress, was utilized to identify 20 medical students experiencing stress.[13] Stress levels were evaluated using both self-reported assessments and the validated DASS-21 scale.[14] Participants completed a questionnaire addressing perceived stress and stress-related symptoms before exam. Individuals with pre-existing medical conditions, obesity, ongoing dental treatment and elderly participants were excluded from the study. This study was approved by Kırşehir Ahi Evran University Faculty of Medicine, Health Sciences Scientific Research Ethics Committee (dated 30.04.2024 and numbered 2024-09/69).

Sample Collection

The students with a mean age of 22 years (range, 20–24 years) participated in the study were chosen using a convenience sampling method. Participants abstained from consuming any food or beverages, except for water, for one hour prior to the collection. Saliva samples (3ml) were collected both before and after the first semester examinations from same individuals and delivered to the laboratory within one hour and subjected to two-step centrifugation. Samples were first centrifuged at 2000g for 15 min to remove any cellular debris, then centrifuged at 2000g for 10 min. Samples were stored at −80°C until cfDNA isolation.[15]

cfDNA Isolation

cfDNA was isolated with magnetic beads, using ZipPrime SafeCAP Cell-Free DNA Extraction and Capturing Kit (ZipPrime, Turkey) according to the manufacturer's protocol. Isolated cfDNA were stored at −80°C until quantification.

Quantification of cfDNA

Isolated cfDNA was first quantified via Jenway Genova Nano Micro-Volume Life Science Spectrophotometer (Cole-Parmer, US). Samples were briefly vortexed and centrifuged before quantification using 2 µL of DNA. Fragment size of cfDNA was assessed using the Agilent 2100 Bioanalyzer (Agilent Technologies, US) with the High Sensitivity DNA kit (Agilent Technologies, US), following the manufacturer's instructions. The predominant fragment size was identified as the peak with the highest molar concentration in the bioanalyzer output.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 10 software (US). Data were presented as means ± standard deviations and significance of differences were analysed using the t test for spectrophotometry results and the nonparametric Mann-Whitney test for Bioanalyzer data.[16] The value of $p \leq 0.05$ was considered statistically significant. Error bars represent technical replicates.

RESULTS

In this study, twenty saliva samples were collected both before and after the first semester examinations from same individuals identified as stressed based on the DASS-21 questionnaire. After cfDNA isolations, amounts were quantified using spectrophotometry initially. Data showed cfDNA levels were significantly elevated in 80% of the participants before examinations ($p \leq 0.05$) (Figure 1).

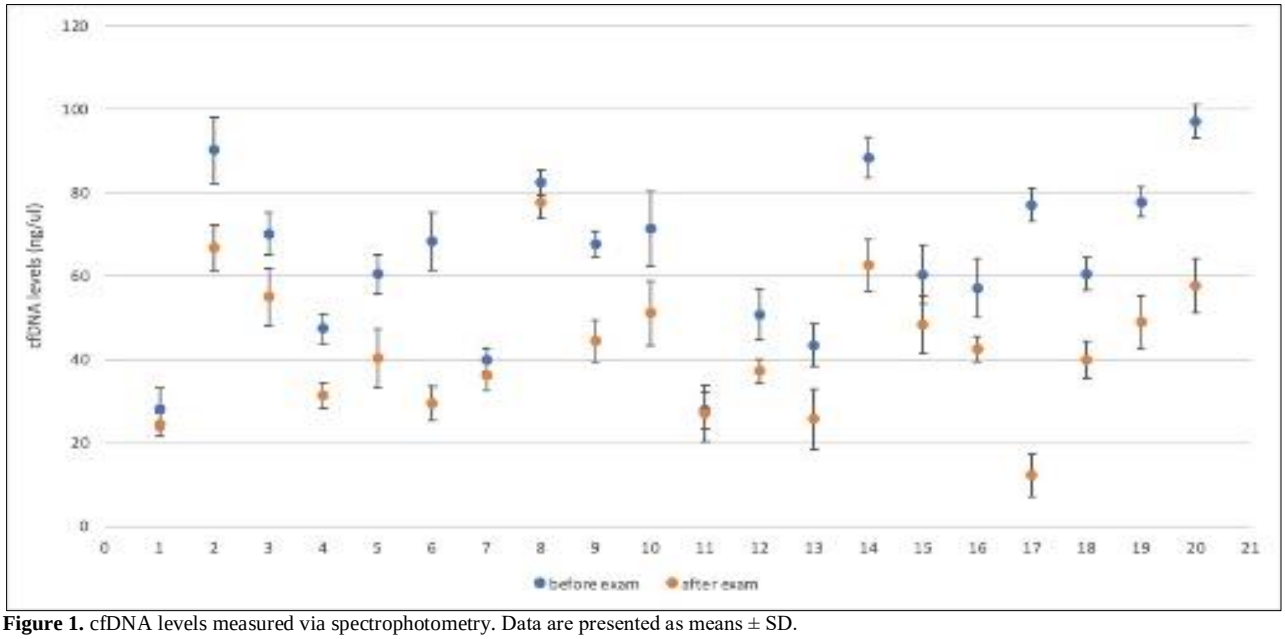

Figure 1. cfDNA levels measured via spectrophotometry. Data are presented as means ± SD.

Since spectrophotometric analysis cannot determine the lengths of cfDNAs, all isolated cfDNAs were quantified collectively after isolation for each sample. Although these results offer a general basis for comparison, they are insufficient for making definitive conclusions. To accurately measure cfDNAs based on their length, we conducted an analysis using automated electrophoresis.[17] In the second phase of the experiment, samples were subjected to Bioanalyzer analysis. Bioanalyzer results showed a significant decrease in all samples correlated with stress elimination ($p \leq 0.05$). cfDNAs range between 40-200 bp were taken into consideration. Data revealed a more than twofold decrease in all samples after examinations, results are shown in Figure 2. A representative Bioanalyzer graph is also added as Figure 3.

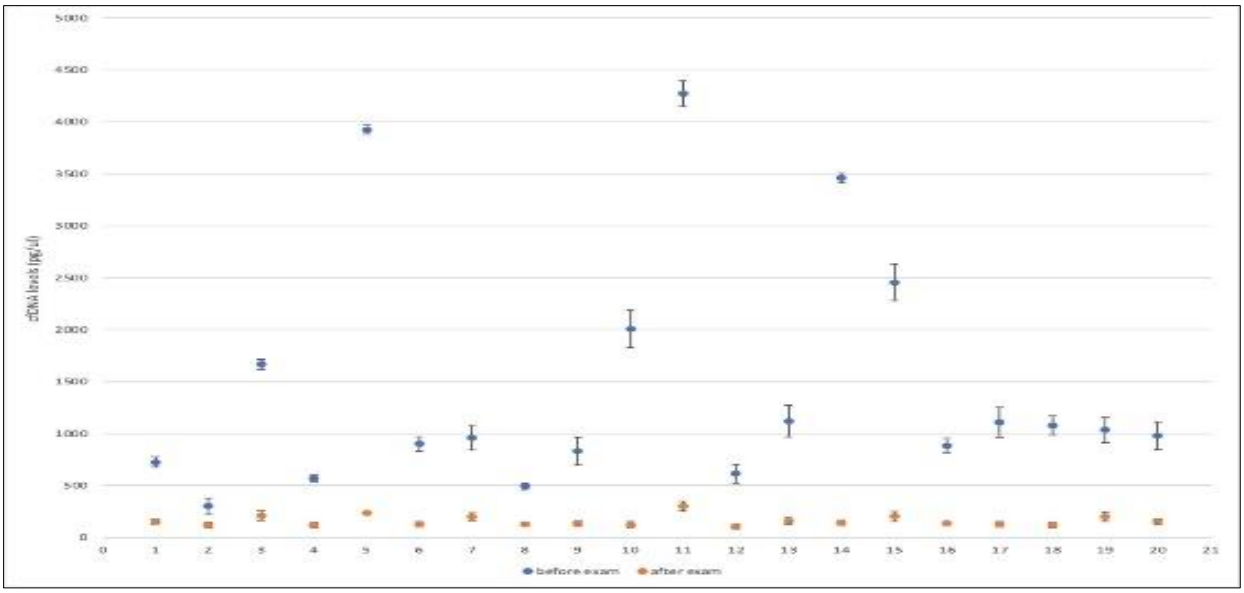

Figure 2. cfDNA levels measured via Bioanalyzer. Data are presented as means ± SD.

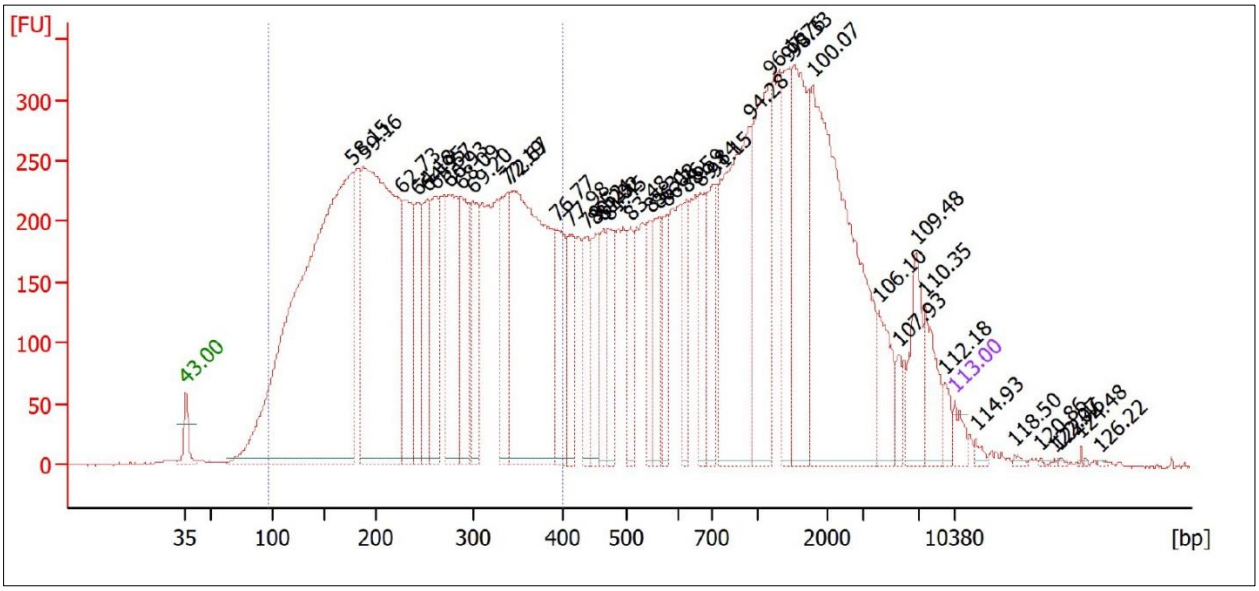

Figure 3. Representative bioanalyzer graph.

DISCUSSION

The purpose of this preliminary study was to investigate the relationship between cell-free DNA (cfDNA) levels and stress. The study included 20 medical students identified as stressed according to DASS-21 scale, whose saliva samples were analyzed for cfDNA levels both before and after the stress-inducing event, which were exams. Data quantified with spectrophotometric analysis (Jenway Genova Nano Micro-Volume Life Science Spectrophotometer) and assessed via automated electrophoresis (Agilent 2100 Bioanalyzer). The results showed elevated cfDNA levels in saliva samples before the exams, cfDNAs whose length between 40-200 bp were taken into consideration ($p \leq 0.05$). Our findings were consistent with the literature.[12]

In addition, our data also showed that participants had longer cfDNAs within the ranges of 500-10.380 bp and 35-10.380 bp (data not shown). These cfDNAs were excluded from the quantification data due to the possibility that they may have originated from the oral microbiome.[18]

Moreover, additional analysis is necessary to ascertain the source of the isolates within the specified range. Although this preparatory study needs additional analysis to draw definitive conclusions, it indicates that stress may impact cfDNA levels in saliva, suggesting the potential use of cfDNA as a biomarker for stress.

Conflict of Interest

The authors declare that there is not any conflict of inter-est regarding the publication of this manuscript.

Ethics Committee Permission

This study was approved by Kırşehir Ahi Evran University Faculty of Medicine, Health Sciences Scientific Research Ethics Committee (dated 30.04.2024 and numbered 2024-09/69).

Authors' Contributions

Concept/Design: EÇ, MBK, HMA, SAA, YEK, CO, SÇ. Data Collection and/or Processing: MBK, KMA, SAA, YEK, CO. Data analysis and interpretation: AÇ, SÇ. Literature Search: EÇ, HMA, SÇ. Drafting manuscript: EÇ. Critical revision of manuscript: EÇ, MBK, HMA, SAA, YEK, CO, SÇ. Supervisor: EÇ.

REFERENCES

1. Bronkhorst AJ, Ungerer V, Diehl F, et al. Towards systematic nomenclature for cell-free DNA. Human Genetics. 2021;140(4):565-578.
2. Zhao X, Zhao H, Liu Y, Guo Z. Methods, bioinformatics tools and databases in ecDNA research: An overview. Comput. Biol. Med. 2023;167:107680.
3. Du S, Cao K, Yan Y, Wang Y, Wang Z, Lin D. Developments and current status of cell-free DNA in the early detection and management of hepatocellular carcinoma. J Gastroenterol Hepatol. 2024;39(2):231-244.
4. Huang J, Hong W, Wan M, Zheng L. Molecular mechanisms and therapeutic target of NETosis in diseases. MedComm. 2022;3(3):162.
5. Van der Meer AJ, Kroeze A, Hoogendijk AJ, et al. Systemic inflammation induces release of cell-free DNA from hematopoietic and parenchymal cells in mice and humans. Blood Advances. 2019;3(5):724-728.
6. Hummel EM, Hessas E, Müller S, et al. Cell-free DNA release under psychosocial and physical stress conditions. Transl. Psychiatry. 2018;8(1):236.
7. Jarmuzek P, Kozlowska K, Defort P, Kot M, Zembron-Lacny A. Prognostic Values of Systemic Inflammatory Immunological Markers in Glioblastoma: A Systematic Review and Meta-Analysis. Cancers. 2023;15(13):3339.
8. Noushad S, Ahmed S, Ansari B, Mustafa UH, Saleem Y, Hazrat H. Physiological biomarkers of chronic stress: A systematic review. Int J Health Sci (Qassim). 2021;15(5):46-59.
9. Herhaus B, Neuberger E, Juškevičiūtė E, Simon P, Petrowski K. Kinetics of Plasma Cell-Free DNA under a Highly Standardized and Controlled Stress Induction. Cells. 2023;12(4):564.
10. Czamanski-Cohen J, Sarid O, Cwikel J, et al. Cell-free DNA and telomere length among women undergoing in vitro fertilization treatment. J Assist Reprod Genet. 2015;32(11):1697-1703.
11. Czamanski-Cohen J, Sarid O, Cwikel J, et al. Decrease in cell free DNA levels following participation in stress reduction techniques among women undergoing infertility treatment. Arch Womens Ment Health. 2014;17(3):251-253.
12. Shan MA, Ishtiaq W, Kanwal S, Khan MU, Iftikhar A, Khan S. Cell-free DNA as a potential diagnostic biomarker in academic stress: A case-control study in young adults. Saudi J Biol Sci. 2024;31(3):103933.
13. Lovibond PF, Lovibond SH. The structure of negative emotional states: Comparison of the Depression Anxiety Stress Scales (DASS) with the Beck Depression and Anxiety Inventories. Behav. Res. Ther. 1995;33(3):335-343.
14. Szabo M, Lovibond PF. Development and Psychometric Properties of the DASS-Youth (DASS-Y): An Extension of the Depression Anxiety Stress Scales (DASS) to Adolescents and Children. Front Psychol. 2022;13:766890.
15. Ferrier ST, Tsering T, Sadeghi N, Zeitouni A, Burnier JV. Blood and saliva-derived ctDNA is a marker of residual disease after treatment and correlates with recurrence in human papillomavirus-associated head and neck cancer. Cancer Med. 2023;12(15):15777-15787.
16. Czeiger D, Shaked G, Eini H, et al. Measurement of Circulating Cell-Free DNA Levels by a New Simple Fluorescent Test in Patients With Primary Colorectal Cancer. Am. J. Clin. Pathol. 2011;135(2):264-270.
17. Polatoglou E, Mayer Z, Ungerer V, Bronkhorst AJ, Holdenrieder S. Isolation and Quantification of Plasma Cell-Free DNA Using Different Manual and Automated Methods. Diagnostics. 2022;12(10):2550.
18. Rapado-González Ó, López-Cedrún JL, Lago-Lestón RM, et al. Integrity and quantity of salivary cell-free DNA as a potential molecular biomarker in oral cancer: A preliminary study. J Oral Pathol Med. 2022;51(5): 429-435.