

Effective use of skin microbiome signatures for fingerprint identification

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Abstract

Background: Recent advances have increased the importance of the human microbiome, including the skin microbiome. Despite the hand microbiome research, the factors affecting the composition of the hand microbiome and their personal characteristics are incompletely known.

Objectives: Despite changing environmental factors and personal variation, we aimed to indicate the interpersonal distinction between skin microbiota using simple and rapid molecular methods.

Methods: Over a non-consecutive 10-day period, samples were taken from 10 adult individuals, and ribotyping analysis of the 16S and 23S genes of *S. epidermidis* was performed on each skin sample. Additionally, EcoRI and HindIII enzyme reactions and variable number tandem repeat (VNTR) reactions of *S. epidermidis* obtained from DNA samples were performed. The skin microbiomes of individuals were evaluated along with the microbiome profiles left on the surfaces they touched.

Results: In the environmental samples taken, it has been observed that people preserve their core skin microbiota characters and carry them to their environment. It was determined that the highest similarity rate was 77.14%, and the lowest similarity rate was 31.74%.

Conclusion: Our study showed that the core skin microbiota retains its characteristics and leaves traces in environments. The fact that the personal microbiome remains unchanged despite environmental differences and has characteristic features has shown that it can be used in forensic sciences to distinguish individuals from each other. These results with simple and rapid methods further increased the importance and significance of the study. The findings indicate that personal skin microbiota can provide a significant contribution to criminal investigations by increasing accuracy and reliability, especially in forensic analyses.

KEYWORDS

fingerprint, hand microbiota, ribotyping, *S. epidermidis*, skin microbiota, VNTR

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1 | INTRODUCTION

Our skin is home to millions of bacteria, fungi, and viruses that compose the skin microbiota. These special microbiota regions are defined essentially as the skin, intestine, oral cavity, respiratory tract, and urogenital region.^{1,2} While recent research shows that these specific microbiota regions show the race and ethnicity of the person,³ it is noteworthy that the microbiota forms a unique identity with factors such as lifestyle, habitat, or dietary behavior of individuals.⁴ Despite the different lifestyles of individuals, the fact that each individual has characteristic signatures of the hand microbiota has shown its use in different areas.^{5,6} It is emphasized that distinguishing the microbiota transferred from the skin of people to the surfaces of the microbiota samples on the environmental surfaces of the person may play a role in forensic identification similar to fingerprints.⁷⁻⁹ It has been shown that individuals can be identified by the microbiota features of unchanged personal microbiota, traces left on computer keyboards, mobile phones, and surfaces touched at home.^{6,10,11}

Although fingerprints are the most frequently obtained evidence in forensic science, it is not always possible to make an identification.¹² The use of microbiota, which carries significant personal and regional differences, has introduced a new perspective in forensic science.^{13,14} Particularly, skin microbiota has guided our study by confirming Locard's Exchange Principle that "every contact leaves a trace." Despite individual differences, it has been shown that bacteria such as *Propionibacterium acnes*, which are commensally present in the human core skin microbiota, can be used for differentiation and identification among individuals.¹⁵ Similarly, *Staphylococcus epidermidis*, which is commensally present in every skin microbiota, is also a valuable resource for identification in forensic science.¹⁶⁻¹⁹ In our study, we aimed to differentiate between individuals and detect the traces they leave on surfaces using simple, rapid, and low-cost molecular methods targeting *S. epidermidis*. This study focused on identifying these evidence-bearing traces and individual differences with easily applicable methods without the need for extensive infrastructure and high costs.

2 | METHODS

2.1 | Sample collection

All experiments within the study were carried out in compliance with the relevant laws and guidelines, under the ethical standards of the Declaration of Helsinki. The individuals included in the study were carefully selected taking into account the conditions that may affect the skin microbiota. Samples were collected from the index fingers of the right hands of 10 participants and their personal belongings for 10 days. All 10 individuals were healthy at the time of sampling and were between 25 and 55 years of age. While selecting individuals, conditions that may affect microbiota, such as professional characteristics, hand antiseptics, topical antibiotic use, or public transportation, were taken into consideration (Table 1). Hand washings of the individuals were requested to be minimally done nine times during a day. Five of

these 10 individuals shared the same department, except for two out of 10 participants who were hospital workers. Sample collection was carried out on non-periodic days and at any time of the day. Also, comparing the bacterial communities on the keyboards that are used by the participants of this study to other finger microbiotas, we swabbed space bar keys from three laptop keyboards located at the University of Istanbul-Cerrahpasa and three other private and public computer keyboards as controls. Skin surfaces and keyboard keys were sampled using sterile cotton-tipped swabs with a sterile solution.^{4,20} Swabbing has been shown to be a suitable method for skin sample collection for microbial community analysis.^{19,21} Swabbing has been shown to be a suitable method for collecting skin samples for microbial community analysis. In our study, the profile of commensal *S. epidermidis* and the entire hand microbiota found on each individual's skin were selected for identification. Simple, easy, low-cost, and rapid molecular methods were used to distinguish between personal skin microbiota and the microbiota left on surfaces touched by individuals. VNTR analysis was applied for typing *S. epidermidis*, and bacterial 16S and 23S enzyme digestion was performed to evaluate the entire skin microbiota profile. The use of both methods and their combined analysis were determined to enhance reliability and sensitivity for the phylogenetic analysis of personal skin microbiota and traces transferred to surfaces.

2.2 | DNA extraction, VNTR, and RFLP assay

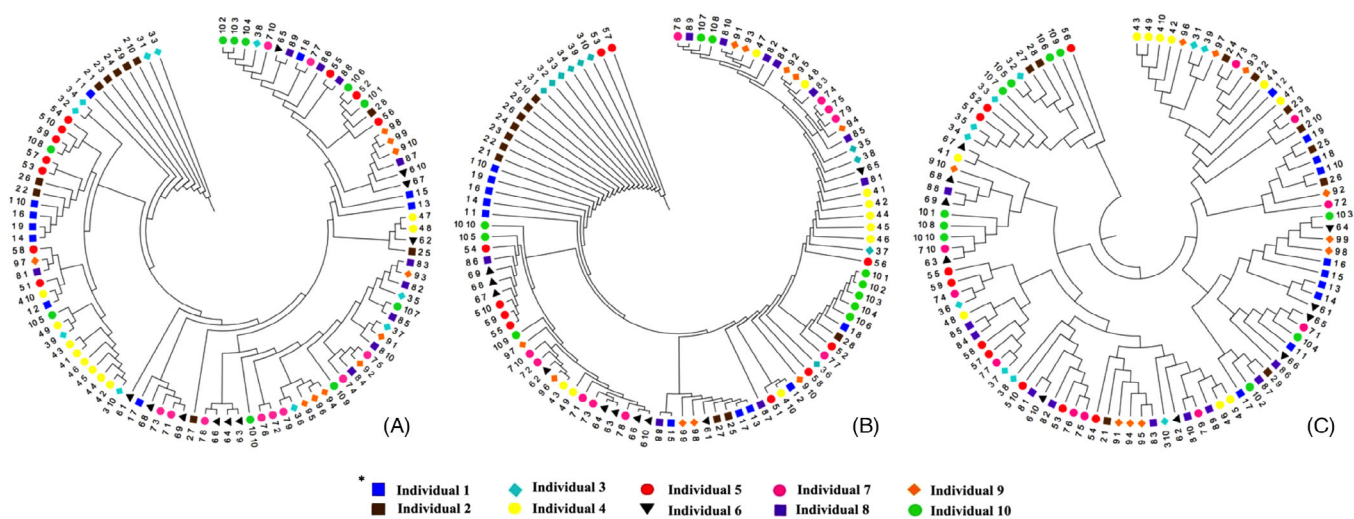
Bacteria belonging to individuals were enriched in tryptic soy agar medium, and DNA from those bacteria was isolated and stored at -20°C until use. Samples were centrifuged at 14 000 rpm for 1 min. The supernatant was discarded, and 250 μL of 1X PCR Buffer was added to the pellet. After a short period of vortexing, centrifugation was done again at 14 000 rpm for 1 min, and the supernatant was discarded. Two hundred fifty microliter of 1X PCR buffer was added to the samples, and a short-term vortex was repeated. The samples were boiled at 100°C for 10 min and centrifuged at 14 000 rpm for 1 min. DNA in the supernatant was stored at -20°C until the PCR. PCR reactions were performed with primers designed specifically for the amplification of 16S and 23S gene regions of the bacterial genome and the target region of *S. epidermidis* for VNTR analysis (for each sample, we amplified 16S rRNA genes using the primer set described in Peerayeh et al. and Francois et al.).^{22,23}

2.3 | Phylogenetic and statistical analysis

A comparison was made with the samples taken from the keyboards of the first three people's personal computers, along with their hand flora. In addition, the 16S, 23S RFLP, and *S. epidermidis* VNTR regions of personal computer samples were investigated. The 'IMAGE LAB' program was used to display the VNTR and cut reactions of *S. epidermidis* and to determine the bands. As a result, PCR and restriction digestion were compared to the 100-bp ladder in the 'IMAGE LAB' program, and their molecular size was determined. VNTR samples of 16S rRNA, 23S rRNA,

TABLE 1 Features of the individuals.

Individual number	Sex	Age	Profession	Known disease	Continuous antibiotic usage	Number of hand washes per day	Specific conditions that can affect hand flora
Individual 1	M	52	Faculty Member	None	No	13–14	Frequent use of liquid hand antiseptics,
Individual 2	M	40	Faculty Member	None	Yes	9–10	Frequent use of liquid hand antiseptics, Use of topical antibiotics to treat acne
Individual 3	F	38	Student, Lab worker	None	No	10–11	Frequent use of liquid hand antiseptics, lab worker
Individual 4	F	30	Lab worker	None	No	10–11	Infection-Bacteriology laboratory working environment
Individual 5	F	26	Student	None	No	9–10	Using metrobus for transportation
Individual 6	F	29	Lab worker	None	No	13–14	Frequent hand washing
Individual 7	M	53	Cleaning staff	None	No	13–14	Mostly working outside
Individual 8	F	34	Cleaning staff	None	No	Least 15	Continuous use of detergents as cleaning staff
Individual 9	M	34	Lab worker	None	No	9–10	Isolated working environment
Individual 10	M	29	Doctor	None	No	11–12	Active patient examination in the outpatient clinic

**FIGURE 1** Phylogenetic dendrogram of VNTR reactions of (A) 16S, (B) 23S, and (C) *S. epidermidis* of the individual's hand microbiota.

and *S. epidermidis* were analyzed in the 'IMAGE LAB' program, and their results were evaluated for three different parameters in the 'FreeTree', 'MEGA7', and 'PRIMER V7' phylogenetic analysis programs (Figure 1).

We used the UniFrac metric to determine the amount of distance between any pair of bacterial communities. UniFrac distances are based on the fraction of branch length shared between three communities within a phylogenetic tree constructed from the 16S rRNA, 23S rRNA, and *S. epidermidis* amplicons from all communities compared.^{24,25} The generated data were evaluated in 'FreeTree', 'MEGA7', and 'PRIMER V7' phylogenetic analysis programs. According to the phylogenetic diagram distance method principle, 'Neighbor

Joining', 'Bootstrapping', and 'Repetition Count' values were arranged as 1000.

3 | RESULTS

Microbiome samples were collected from the right index finger of 10 participants on 10 different days to analyze the hand microbiomes. Contributors were chosen as two faculty members, two students, two cleaning staff, three laboratory staff working in different fields, and a clinician. The individuals participating in our study are mostly hospital

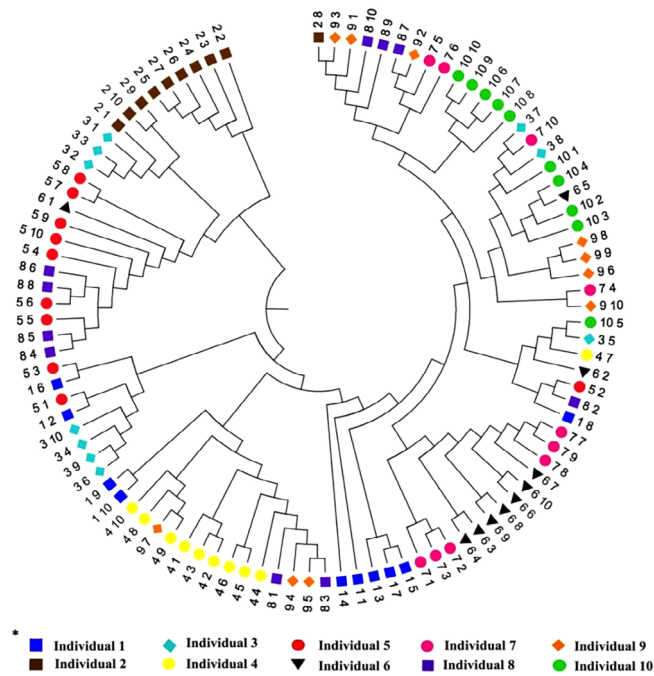


FIGURE 2 Phylogenetic dendrogram in which the VNTR reactions of 16S, 23S, and *S. epidermidis* belonging to the hand microbiota of individuals are evaluated together.

employees; the use of hand antiseptics and hand wash count has been identified as minimally nine times. Individual #1 is an academic member of the infectious diseases group. Along with being in the areas that may affect the microbiota, individual #1 frequently washes hands and uses hand antiseptics. Despite these factors, individual #1's microbiota samples were found in the same root in the dendrogram. Likewise, individual #2, who is a member of the faculty, used topical antibiotics during sample collection. Despite this factor, which may affect the hand microbiota, it has been found that this person's core hand microbiota has not changed. The individual #5, who is a student, touched different surfaces while using public transportation. Similarly, individuals #7 and #8, who are cleaning staff in different departments, and the medical doctor working in the clinic have also shown that they protect their unique and core hand microbiota. Although individuals #3, 4, 6, and 9 are different laboratory workers, it was shown that the core hand microbiota is unchanged in those participants (Figure 2).

Furthermore, in our study, the personal desktop computers of three people were compared with their core hand microbiota. To increase the distinction power in microbiota samples taken, VNTR analysis of *S. epidermidis* was performed with 16S rRNA and 23S rRNA gene regions by using EcoRI and HindIII restriction enzyme digestion. Our results demonstrated that the core hand microbiota does not change; it is transferred to inanimate surfaces and also carries its characteristics onto personal belongings.

When the results were evaluated in phylogenetic analysis programs, although the hand microbiota of each individual differed from each other, it was remarkable that there was a transition between the core hand microbiota of people working together. Although the hand micro-

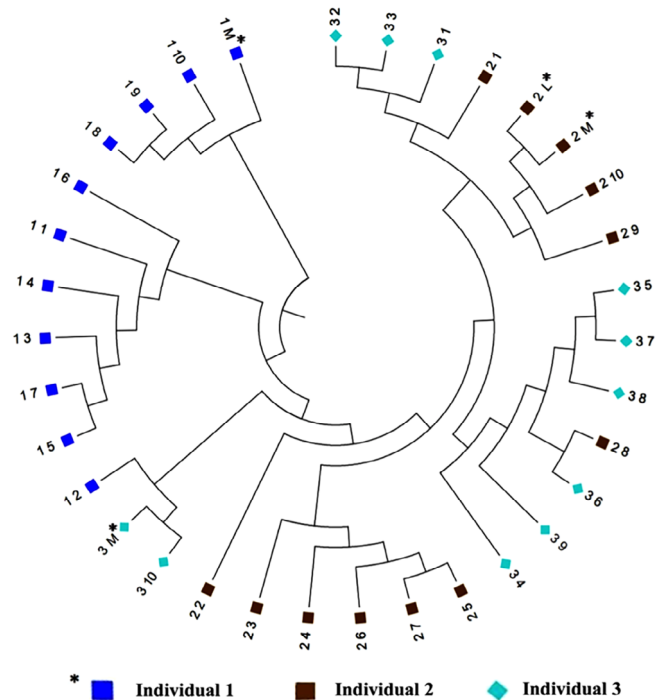


FIGURE 3 Phylogenetic dendrogram in which 16S, 23S, and *S. epidermidis* VNTR are evaluated together with hand microbiota and keyboard samples taken from personal computers belonging to first, second, and third individuals (M* = keyboard sample of desktop computer, L* = keyboard sample of laptop).

biota of individuals #2 and #3 shows significant differences, transfer to the surface they contacted took place. On the other hand, individual #1's microbiota was again completely identical to his personal computer, while individuals #2 and #3 were excluded (Figure 3).

Another evaluation in our study was made according to the working environments of the participants. In our study, the participants were selected from two different departments. The blue and red square symbols in Figure 4 represent departments of people. Four out of 10 people are in the blue area and are shown in blue. Five out of 10 are in the independent G section. In the figure, although each person has a unique microbiome, people in the same section are close to each other. One of the individuals (3MK) is the person in the M and G zones. It was clearly seen that there was a person who had contact with both departments and that he had traces from both departments. Although individuals have unique characteristics in terms of hand microbiota, it has been found that each person has characteristic traces specific to the working environment (Figure 4).

In order to increase the discrimination power, 16S rRNA, 23S rRNA, and VNTR data of *S. epidermidis* were evaluated together, and 12 cluster separations were achieved. While the interpersonal similarity rate was found to be 77.14% at the most, the lowest similarity rate was determined to be 31.74% (Table 2). According to the results of this study, interpersonal coverage or exclusion was achieved by phylogenetic analysis of hand microbiota samples that can be considered invisible evidence. In this case, our results showed that the core hand microbiota sample can be used as evidence in forensic science.

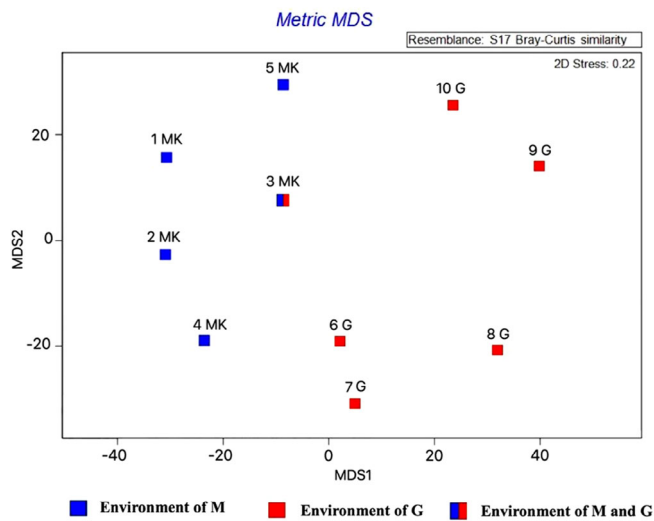


FIGURE 4 Evaluation of hand microbiota samples by 'Bray-Curtis' similarity analysis according to the environments of the people.

4 | DISCUSSION

The main objectives of the Human Microbiome Project are to identify all microorganisms, reveal microbiome differences between humans, and investigate the relationship between the microbiome. Using the personal skin microbiome as silent evidence in the field of forensic microbiology can explain the case, and recent developments bring different perspectives.¹²

Although fingerprints are important as basic evidence, there are cases where fingerprint evidence is insufficient. The perpetrator often removes fingerprints by sanding, burning, or applying chemical damage to avoid leaving marks. Furthermore, in cases where the fingerprint is fragmented, a print may not contain enough comparison points. However, in cases where DNA and fingerprints are absent or insufficient, the case can be explained by the skin microbiota released into the environment.^{26,27} In the case of the detection of microorganisms, primary and even secondary death sites can be distinguished by comparing the microbiota of the victim and the environment. In addition,

results from the microbiome found on the victim may explain the link between the case and possible suspects.¹³

The study by Fierer et al. aimed to use the diversity of skin-related bacterial communities and individual differences in forensic identification. They tried to distinguish between the microorganisms remaining on objects by contact or even samples taken from personal items that have been untouched for 2 weeks. The samples from nine computer keyboards and mouse used for a maximum of 12 h were compared with the palm flora, and each sample was shown to be significantly similar to the user.⁶ Also, Schmedes et al. took 14 different samples from 12 healthy human bodies for 2.5 years to study their skin microbiome. In this particular research, the authors aimed to create a microbiome profile by evaluating 187 different microorganisms. The samples were taken three times a day. Especially, the SNP distinction power of *Propionibacterium acnes* has increased. In the long-term study, how much the microbiota varied was compared.²⁶ In another study, the topographic diversity of microorganisms belonging to the skin microbiota was evaluated using phylogenetic analysis of the 16S rRNA gene region. While most identified breeds constitute *Corynebacterium*, *Propionibacterium*, and *Staphylococcus*, the density of each depends on the nature of the species. For example, while *Propionibacterium* and *Staphylococcus* species were dominant in the oily areas on the face, *Corynebacterium* species predominated in humid areas such as axilla. Even *Staphylococcus* species were present.²⁸

The hand microbiota is more variable than in other skin regions. However, it was determined that hand microbiota diversity decreased as a result of washing hands with soap more than 40 times or using hand antiseptics. It has been shown that the stable and characteristic microbiota regains its properties a few hours after hand washing.²⁷ In the absence of biological evidence such as blood, tissue, semen, saliva, or post-mortem samples, which are important pieces of evidence in forensic science, specific microbiota samples constitute a different source of evidence.²⁹⁻³⁰ Any evidence from the crime scene is important for the comparison, exclusion, and matching of individuals. Today, with the development of technology, different ideas, and techniques are being developed to destroy evidence in planned or unplanned crimes. The association with the personal microbiota has provided a

TABLE 2 Evaluation of similarity ratios of individuals' microbiota samples in PRIMER V7.

Similarity (0-100)									
1 MK									
2 MK	70.17								
4 MK	60.31	66.67							
3 S	55.38	58.06	55.82						
5 G	61.29	61.01	64.61	53.73					
6 G	65.57	65.51	56.25	48.45	60.31				
8 G	58.46	61.29	58.82	77.14	62.68	57.57			
7 G	45.45	44.44	49.27	61.97	41.17	47.76	61.97		
9 G	31.74	40.01	42.44	47.05	52.30	52.30	47.05	55.07	
10 G	50.79	46.66	54.54	50.32	46.15	46.15	47.59	52.17	72.72

different perspective in response to efforts to remove any remaining fingerprints or DNA traces.^{13,28,31,32}

In our study, we have shown that microorganisms that cannot be detected by classical culture methods can be used effectively by molecular methods and that much more detailed data can be obtained with minimum cost. Furthermore, we determined that the core hand microbiota does not change depending on the living conditions of the people and the count of hand washes. We found that although there are a wide variety of microorganisms in environments that may affect the microbiota, such as using public transportation or having duties in infection laboratories or outpatient clinics, people do not lose their own traces of the microbiota. In samples taken from personal computers that we evaluated in our study, we determined that microorganisms migrated from living things to surfaces and preserved their properties for a certain period of time. In addition, we have determined that each person has microbial traces in their own working environment. Our results described the characteristics of the biogeographic environment in which the samples were collected, together with the core microbiota characteristics. Accordingly, the hand microbiota samples of the people in two different environments were compatible with their work environments, and the distinction between the two groups was successfully demonstrated. Even though fingerprint analysis and DNA identification are the most common methods in forensic science, insufficient, contaminated, or gradient sample problems are frequently encountered in the evaluation process. In such cases, the examination of the same or different evidence material by using a different method is used to explain the event. In our study, ribotyping of 16S and 23S gene regions and VNTR analysis of *S. epidermidis* were evaluated on hand microbiota samples. Besides, while the interpersonal similarity rate was 77.143% at most, the lowest similarity rate was found to be 31.74%. According to our findings, interpersonal coverage or exclusion was achieved by phylogenetic analysis of hand microbiota samples, which provides invisible evidence.

As technology develops, different methods and techniques are being developed to eliminate evidence of planned or unplanned crimes. In response to efforts to remove any fingerprints or DNA traces left behind, we have provided a different perspective on the characteristic microbiota.

In conclusion, it has been demonstrated that identification based on microbial traces from individuals can be achieved using simpler, faster, and more cost-effective methods without the need for advanced technologies. Instead, the results of our study were obtained using methods that are independent of infrastructure and kits. It has been proven that the microbiota transferred to surfaces by individuals and their core microbiota are preserved and personal despite all environmental variables. The study also indicates that these personal traces, which include commensal bacteria (such as *P. acnes* and *S. epidermidis*), could become significant forensic tools when tested with larger sample sizes and when similar effects are confirmed through repeated studies. Accordingly, the potential of microbiome-based findings to contribute to solving crimes has been demonstrated. Finally, our study has shown that by increasing sample sizes and evaluating multiple microorganisms, the accuracy and reliability of skin micro-

biota as strong evidence of individuality in forensic sciences can be enhanced.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

All samples were obtained after informed consent and with the approval of the Istanbul-Cerrahpaşa University Faculty of Medicine Ethics Committee [346298]. All experiments within the study were carried out in compliance with the relevant laws and guidelines, under the ethical standards of the Declaration of Helsinki.

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