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Journal of Immunology and Clinical Microbiology

ORIGINAL ARTICLE / ÖZGÜN MAKALE

Evaluation of Antibiotic Consumption in Hospital: Single-Center Point Prevalence Study

Hastanede Antibiyotik Tüketiminin İncelenmesi: Tek Merkezli Nokta Prevalans Çalışması

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Abstract

Objective: The purpose of the research is to evaluate antibiotic consumption in hospitalized patients with a single-day point prevalence study.
Material and Methods: The study was conducted at Yalova Training and Research Hospital (YTRH) on April 12, 2023. The research day, patients in all clinics and intensive care units were visited, and information about the sociodemographic data of the patients and the antibiotics used was recorded in the antibiotic consumption form. The resulting box-based consumption was analyzed in the SPSS program in the Defined Daily Dose (DDD) unit of the World Health Organization.
Results: 150 patients included in the research, and 100 patients received antibiotics. Antibiotic use rate was found to be 66.66%. 55 of the patients receiving antibiotics were female and the mean age was 66.36±18.54 (min:19, max:92) years. The total amount of antibiotics consumed was 839.60 DDD/1000 patient-days (DPD). When antibiotic consumption was evaluated according to units, it was seen that the patients using antibiotics the most were in surgical wards (n: 47; 227.4 DPD), and the highest antibiotic consumption was in intensive care units (ICU) (n: 29; 399.83 DPD). The first three antibiotics most used in the hospital were moxifloxacin, ceftricasone and ciprofloxacin, respectively.
Conclusions: The study found that 66% of patients used at least one antibiotic, the most commonly used units were the ICU, and the most commonly used antibiotic groups were quinolones, cephalosporins and carbapenems. Antibiotic consumption should be monitored using the point prevalence method, feedback should be provided and awareness should be raised by implementing antibiotic stewardship programs in the hospital.
Keywords: Antibiotic, Antibiotic consumption, Point prevalence

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Öz

Amaç: Bu çalışmanın amacı, hastanede yatan hastalarda antibiyotik tüketimini tek günlük nokta prevalans çalışmasıyla değerlendirmektir.
Gereç ve Yöntem: Çalışma, 12 Nisan 2023 tarihinde Yalova Eğitim ve Araştırma Hastanesi›nde (YEAH) gerçekleştirildi. Araştırma günü, tüm servis ve YBÜ'deki hastalar ziyaret edilerek hastaların sosyodemografik verileri ve kullanılan antibiyotiklere ilişkin bilgiler antibiyotik tüketim formuna kaydedildi. Elde edilen kutu bazlı tüketim, Dünya Sağlık Örgütü›nün Günlük Tanımlanmış Doz (GTD) biriminde SPSS programında analiz edildi.
Bulgular: Araştırmaya 150 hasta dahil edildi ve 100 hastanın antibiyotik aldığı tespit edildi. Antibiyotik kullanım oranı %66,66 olarak belirlendi. Antibiyotik kullanan hastaların %55'i kadın olup yaş ortalaması 66,36±18,54 (min:19, max:92) yıl idi. Tüketilen toplam antibiyotik miktarı 839,60 GDD/1000 hasta-günü (GHG) idi. Birimlere göre antibiyotik tüketimi değerlendirildiğinde en fazla antibiyotik kullanan hastaların cerrahi servislerde (n:47; 227,4 GHG) olduğu, en fazla antibiyotik tüketiminin ise yoğun bakım üniteleri (YBÜ)'nde (n:29; 399,83 GHG) olduğu görüldü. Hastanede en çok kullanılan ilk üç antibiyotik sırasıyla moksifloksasin, seftriakson ve siprofloksasin oldu.
Sonuç: Araştırmada hastaların %66'sının en az bir antibiyotik kullandığı, en çok kullanılan birimlerin YBÜ olduğu, en çok kullanılan antibiyotik gruplarının ise kinolonlar, sefalosporinler ve karbapenemler olduğu belirlendi. Nokta prevalans yöntemiyle antibiyotik tüketimi izlenmeli, geri bildirim sağlanmalı ve hastanede antibiyotik yönetim programları uygulanarak farkındalık artırılmalıdır.
Anahtar Kelimeler: Antibiyotik, antibiyotik tüketimi, nokta prevalans

INTRODUCTION

Antibiotics, which have contributed to the treatment of diseases and paved the way for modern medicine for more than 100 years with the discovery of antibiotics by Sir Alexander Fleming in the 1900s, are used in many medical disciplines such as the treatment of infectious diseases, organ transplantation, rheumatology, oncology, invasive procedures and pre-surgery (1-3). However, over time, inappropriate and long-term use of antibiotics has become an important public health problem that poses a global threat of antibiotic resistance. Many microorganisms can become resistant to antimicrobial drugs currently in use by developing more than one resistance mechanism. With the development of antimicrobial resistance, problems such as the increase in resistant bacterial infections, increased occurrence of antibiotic side effects, triggering an increase in morbidity and mortality, and consequently an increase in the economic burden on healt (4,5).

Frequent and excessive use of antibiotics is a global health problem in low- and middle-income countries where access to quality healthcare is limited and regulations on antibiotic use are inadequate (6). According to the report of the World Health Organization (WHO) on "Antibiotic use in Eastern European countries", Turkey is among the countries with high total antibiotic use. Legal regulations are made in our country regarding the rational use of antibiotics, which is called giving antibiotics to the right patient, for the right indication, for the right time and in the right dose range, and with the law enacted in 2014, antibiotics are administered only with a physician's prescription. However, despite these regulations, there are scientific reports indicating that a large proportion of antibiotics used in treatment are inappropriate use. Inappropriate antibiotic

use, especially in inpatients, causes the emergence of resistant microorganisms throughout the hospital, increases mortality and morbidity, and increases the length of hospital stay (7,8).

Conducting point prevalence studies on antibiotic use in hospitals is a guiding surveillance method in determining antibiotic use situations. The aim of this study is to evaluate antibiotic consumption in hospitalized patients with a single-day point prevalence study.

MATERIAL AND METHODS

Study design

This study, planned as a single-day point prevalence study, was conducted at Yalova Training and Research Hospital (YTRH). YTRH is a tertiary care hospital with a total of 400 beds, 100 of which are intensive care unit (ICU) beds. Ambulatory patients were excluded. All inpatients visited between 08:00 and 17:00 pm on the day of the study in each department concerned. An antibiotic consumption form (ACF) was prepared before the study, and the inpatient services and intensive care units in the hospital were visited by the study team on the study day (April 12, 2023). ACF consisted of demographic data and information on antibiotic use. Antibiotic consumption (in milligrams) in ICU, internal wards and surgical wards was recorded in ACF.

Calculation of antibiotic consumption

Antibiotic use was calculated by the 'Anatomical Therapeutic Chemical/Defined Daily Dose (ATC/DDD)' method suggest that the WHO and explained as DDD/1000 patient-days (DPD).

Statistics

Point surveillance data were recorded on the point surveillance form created by the researchers. Data analysis was done SPSS 22 for Windows (Statistical Package for Social Science, SPSS® Corp., Armonk, NY, USA). Categorical variables will be displayed with frequency and percentage and analyzed with the chi-square test. Continuous variables were first tested for normality using the Shapiro-Wilk test. Continuous variables complying with normal distribution were shown with arithmetic mean±standard deviation and minimum and maximum values and were evaluated with the t-test. Continuous variables that did not comply with normal distribution were shown as median, first and third interquartile range values and analyzed with the Mann-Whitney test. Evaluation of two consecutive measurements was done using the Wilcoxon test (paired samples). The significance level was taken as <0.05 (confidence interval 95%).

Ethical considerations

Ethics approval for the study was taken from Yalova University Human Research Ethics Committee with protocol number 2023/109. All participants were informed before the study and were included in the study after their consent was obtained. The study was conducted in accordance with the principles of the Declaration of Helsinki.

RESULTS

150 patients hospitalized in various departments were included in the study and it was seen that 100 patients received antibiotics. Analyzes were performed on a total of 100 patients (29 in intensive care units, 24 in internal services and 47 in surgery services) data. The median age was 66 years, with the largest group aged between 18 and 92. The sex ratio was 0.81, with 55 (55%) female subjects and 45 (45%) male subjects. 74% of the patients had at least one chronic disease. The median hospitalization day of the patients was 3.50 [2.00-08.00] days. 38% of patients had undergone surgery, and the median postoperative hospital stay was 2.0 [1.00-6.00] days.

The antibiotic use rate was found to be 66.6%, and 55% of the patients receiving antibiotics were receiving single antibiotics (144.25 DPD), 33% were receiving double antibiotics (380.70), 8% were receiving triple antibiotics (190.55 DPD) and 4% were receiving quadruple antibiotics (124.09 DPD) (p=0.000). The frequency of antibiotic receive according to hospital departments is shown in Table 1. Total antibiotic consumption in the study day was 839.6 DPD.

When antibiotic consumption is evaluated according to units; It was observed that the patients who used the most antibiotics were in surgical clinics (n: 47, 83.9%, 227,4 DPD) and the highest amount of antibiotic use was in intensive care units (n: 29, 69.0%, 399.83 DPD) (Table 2). The first three antibiotics most used in the hospital were moxifloxacin, ceftriaxone and ciprofloxacin respectively (Figure 1). The most frequently used antifungal was fluconazole (Figure 1).

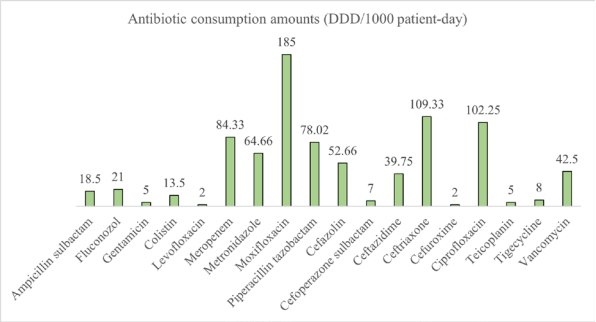

Figure 1. Antibiotic and antifungal consumption amounts (DDD/1000 patient day)

Table 1. Data on patients' demographic information and antibiotic consumption information

	n/mean (min-max)/ median [Inter-quartile range]
Inpatient	150
Patient taking antibiotics	100
Antibiotic use rate %	66.6
Age	66.3 (18.0-92.0)
Gender	
Female	55
Male	45
Chronic disease	
Yes	74
No	26
Hospital admission day	3.50 [2.0-8.0]
Patient having surgery	38
Postoperative hospital day	2.0 [1.0-6.0]
Distribution of patients receiving antibiotics	
Patient receiving single antibiotics	55
Patient receiving double antibiotics	33
Patient receiving triple antibiotics	8
Patient receiving quadruple antibiotics	4

Table 2. Antibiotic use prevalance in intensive care units and services

	Intensive care units n: 42	Internal services n: 52	Surgery services n: 56	P value
Number of patients using antibiotics	29 (69.0)	24 (46.1)	47 (83.9)	0.000*
Antibiotic consumption amount (DPD)	399.8	212.3	227.4	0.000**

* chi-square test, p<0.05; **Kruskal Wallis test, p<0.05

DISCUSSION

Point prevalence studies in hospitals are widely used to because to determine antibiotic exposure (9-11). In our point prevalence study, we found that the antibiotic use rate was 66.6%. In a multicenter point surveillance study investigating the use of antibiotics in hospitalized patients in Northern Nigeria, it was found that 80.1% of the patients used at least one antibiotic on the day of the research (12). In a multicenter point surveillance study on antibiotic use in Pakistan, it was found that 77.6% of hospitalized patients received antibiotic treatment (13). In a point surveillance study conducted in Benin, the point prevalence of antibiotic treatment was 64.6%, and 30% of uninfected patients were reported to have received antibiotics (14). In a study conducted in public hospitals in Ethiopia, the overall prevalence of antibiotic consumption was found to be 63.8% (15). In a point prevalence study on antimicrobial consumption in Brazilian hospitals, it was found that 52.2% of patients used antibiotics (16). In another point surveillance study conducted in intensive care units in Brazil, the prevalence of antibiotic use was reported

to be 52.4% (17). In a study reported on the use of antibiotics in hospitals of the Ministry of Health in Saudi Arabia, the rate of antibiotic consumption was reported as 46.9% (18). In a point prevalence study conducted in Canadian hospitals, it was shown that 34.0% of inpatients were given antimicrobials (19). In a single-center study conducted to evaluate antibiotic use in Turkey, it was reported that 47.7% of patients received at least one antibiotic (20). In a multicenter point prevalence study conducted to determine antibiotic consumption in Turkish hospitals, it was determined that 44.8% of patients received at least one antibiotic (21). In another point prevalence study conducted in our country, it was determined that 30.3% of patients used antibiotics (22). Antibiotic consumption in hospitals varies from one continent to another, from one country to another, and from one department to another. According to these data, it has been observed that antibiotic use rates in hospitals vary between 30% and 80%. Authors in some countries have attributed this high consumption of antibiotics to the relatively high number of tuberculosis cases and the number of patients living with HIV (immunosuppression) (23). In our study, more than half of the inpatient in our hospital use antibiotics. This situation should be handled carefully, precautions should be taken for rational antibiotic use and antibiotic management programs should be developed.

Since the daily defined doses of antibiotics differ from each other, WHO has recommended the use of the ATC/DDD calculation method to ensure standardization of consumed antibiotics (24). Using the WHO ATC/DDD method, we determined that the amount of antibiotics consumed in our study was 839.6 DPD. In a study that systematically reviewed antibiotic consumption in acute care hospitals, It was founded that the aggregate rate of antibiotic consumption throughout the hospital was 586 DDD for all antibacterials (25). In a study conducted in Vietnam, the average antibiotic consumption of the hospital was found to be 918 DDD/1000 patient-days (26). The average antibiotic consumption in a tertiary hospital in Korea was found to be 920.69 DDD/1000 patient-days (27). The Australian national antimicrobial annual report states that the antibiotic consumption was 883 DPD in the country in 2019 (28). In the multicenter point surveillance study carried out in our country, the mean antibiotic consumption amount was calculated as 674.5 DPD (21). In a study conducted to define antibiotic consumption with the participation of 530 hospitals in France, it was reported that 633 DPD antibiotics were used in teaching hospitals (29). It was determined that the total antibiotic consumption of patients hospitalized in a teaching hospital in Nigeria was 260.9 DDD/100 bed-days (30). In a study investigating antibiotic consumption in a 610-bed, tertiary hospital in Quito, Ecuador, researchers found that the average antibiotic prescription rate in the hospital was 148.8 DDD/100 patient days (31). In the study of Borg et al., in which antibiotic consumption was investigated in Southern and Eastern Mediterranean hospitals, including our country, the median value of antibiotic consumption was reported to be 112 DDD/100 bed-days (32). As seen in the literature, antibiotic use in our hospitals is higher than in some countries and lower than in others. We predict that antibiotic consumption may be related to the antibiotic management programs implemented in countries rather than the development level of the country. We believe that as antibiotic consumption decreases with the implementation of antibiotic stewardship programs, both antimicrobial resistance and related expenses will decrease.

Intensive care units represent the most used antibiotic burden in the hospital and are also considered as a department that creates, spreads and strengthens antibiotic resistance, which is important from a microbiological point of view (33). Serious or life-threatening infections are common in patients in the ICU. Following complicated cases in the ICU, the severity of the infections seen, the complexity of the decision-making process and the employment of physicians with limited antibiotic knowledge make it difficult to implement antibiotic management programs in these

departments. In our study, it was founded that the department that consumed the most antibiotics was intensive care units. The amount of antibiotics consumed in the ICU was 399.8 DPD; This rate is approximately twice as high as in surgical and internal departments. In the study in which antibiotic consumption in acute care hospitals was systematically reviewed, it was determined that the unit where the most antibiotics were consumed was the ICU (1563 DDD) and was approximately twice as high as the mean amount of antibiotics consumed (586 DDD) (25). In a study carried out in a tertiary hospital in Kenya, the frequency of rational antibiotic use was 18.5%; Inappropriate antibiotic selection (51.0%) and wrong duration (32.3%) were determine to be the most widespread irrational practices (34). It is thought that continuous in-service training of physicians working in ICUs in order to increase their antibiotic knowledge and regular surveillance of ICUs can contribute to the rational use of antibiotics in ICUs, both by regulating the antibiotic treatments of patients and by being a role model for physicians working in these departments.

In our study, the most widespreadly consumed antibiotics in the hospital were moxifloxacin, ceftricasone, ciprofloxacin and meropenem. In a multicenter point prevalence study investigating antibiotic consumption among hospitalized patients in Northern Nigeria, it was showed that the most frequently prescribed antibiotics were metronidazole, ciprofloxacin, ceftriaxone, amoxicillin-clavulanate and gentamicin (12). In a point prevalence study investigating the use of antibiotics in Ethiopian public hospitals, the most frequently consumed antibiotic in the hospital was ceftriaxone, followed by metronidazole (13). In a point surveillance survey conducted on antibiotic use in twenty six Saudi hospitals, it was observed that the most frequently prescribed antibiotic group was third-generation cephalosporins (14). In the multicenter point surveillance study conducted by Saleem et al. in Pakistan, the three most frequently prescribed antibiotics were ceftriaxone, metronidazole and ciprofloxacin (13). While the most commonly prescribed antibiotics in Botswana public hospitals are cefotaxime and metronidazole, it has been determined that it is ceftriaxone in private hospitals (35). In the study conducted in Turkish hospitals, it was concluded that the most frequently consumed restricted antibiotics were carbapenems, piperacillin-tazobactam and intravenous quinolones, in the order of use, and one of the most commonly consumption unrestricted antibiotics was cephalosporins (21). In another study from Turkey, the most frequently used antibiotic on the spot surveillance day was fluoroquinolones used by 1,147 patients; This was followed by carbapenems and first-generation cephalosporins, used by 1,127 and 1,088 patients, respectively (22). The results of our study are compatible with the literature.

Study Limitations: The most important limitations of our study can be listed as the fact that it was conducted in a single center and only the amounts of antibiotics consumed were examined without evaluating the suitability of antibiotics for their intended use. However, in our study, no distinction was made between preoperative prophylactic antibiotic use and therapeutic antibiotic use. By paying attention to these points in future studies and planning large-scale multi-center studies, the level of irrational antibiotic use in hospitals can be revealed.

CONCLUSION

As a result, it was observed that more than half of the inpatients in our study used antibiotics, the most frequently used antibiotics were moxifloxacin, ceftricasone, ciprofloxacin and meropenem, and the highest use was in ICUs. The high amount of antibiotic consumption in our hospital is related to the resistant microorganisms seen in our country. Antibiotic management programs that will ensure the rational use of antibiotics should be used in hospitals, antibiotic consumption amounts should be monitored and feedback should be given to the units regarding antibiotic consumption.

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REFERENCES

1. Tokur Kesgin M, Zengin S, Çağlar S. Antibiotic Use in Society: A Section from Bolu Province. Ahi Evran Medical Journal. 2023; 7(2), 145-154.
2. Huemer M, Mairpady Shambat S, Brugger SD, Zinkernagel AS. Antibiotic resistance and persistence—Implications for human health and treatment perspectives. EMBO reports. 2020; 21(12), e51034.
3. Hutchings MI, Truman AW, Wilkinson B. Antibiotics: past, present and futur. Curr Opin Microbiol. 2019;(51):72-80.
4. Nepal A, Hendrie D, Selvey LA, Robinson S. Factors influencing the inappropriate use of antibiotics in the Rupandehi district of Nepal. Int J Health Plann Manage, 2021; 36(1):42-59.
5. Erdeniz EH, Dursun A. Evaluation of inappropriate antibiotic use in pediatric patients: Point-prevalence study. Journal of Pediatric Infection, 2020; 14(2)
6. Giorgia S, Sena S, Sumanth G. How can we tackle the overuse of antibiotics in low- and middle-income countries?. Expert Review of Anti-infective Therapy. 2023; 21:11, pages 1189-1201.
7. Gaygısız Ü, Lajunen T, Gaygısız E. Community Use of Antibiotics in Turkey: The Role of Knowledge, Beliefs, Attitudes, and Health Anxiety. Antibiotics (Basel). 2021; 27;10(10):1171.
8. World Health Organization (WHO). Turkey takes strong action to reduce antibiotic consumption and resistance. Accessed on December 22, 2023. Available from: https://www.euro.who.int/en/health-opics/disease-prevention/antimicrobial- resistance/news/news/2017/11/turkey-takes-strong-action-to-reduce-antibiotic-consumption-and-resistance
9. European Centre for Disease Prevention and Control (2016). Point Prevalence Survey of Healthcare Associated Infections and Antimicrobial Use in European Acute Care Hospitals – Protocol Version 5.3. Stockholm: European Centre for Disease Prevention and Control. Accessed on January 15, 2024 Available from: https://www.ecdc.europa.eu/sites/default/files/media/en/publications/Publications/PPS-HAI-antimicrobial-use-EU-acute-care-hospitals-V5-3.pdf
10. Centers for Disease Control and Prevention (2021). Healthcare-Associated Infections - Community Interface (HAIC). HAI and Antibiotic Use Prevalence Survey. Centers for Disease Control and Prevention. Accessed January 15, 2024 Available from: https://www.cdc.gov/hai/eip/antibiotic-use.html
11. Global (2022). Point Prevalence Survey of Antimicrobial Consumption and Resistance (Global-PPS)Why a Global-PPS? University of Antwerp bioMérieux. Accessed January 15, 2024 Available from: https://www.global-pps.com/project/
12. Abubakar U. Antibiotic use among hospitalized patients in northern Nigeria: a multicenter point-prevalence survey. BMC Infect Dis. 2020;30;20(1):86.
13. Saleem Z, Hassali MA, Versporten A, Godman B, Hashmi FK, Goossens H, et al. A multicenter point prevalence survey of antibiotic use in Punjab, Pakistan: findings and implications. Expert Rev Anti Infect Ther. 2019;17(4):285-293.
14. Ahoyo TA, Bankolé HS, Adéoti FM, Gbohoun AA, Assavèdo S, Amoussou-Guénou M, et al. Prevalence of nosocomial infections and anti-infective therapy in Benin: results of the first nationwide survey in 2012. Antimicrob Resist Infect Control. 2014;14;3:17.
15. Fentie AM, Degefaw Y, Asfaw G, Shewarega W, Woldearegay M, Abebe E, et al. Multicentre point-prevalence survey of antibiotic use and healthcare-associated infections in Ethiopian hospitals. BMJ Open. 2022;11;12(2):e054541.
16. Porto APM, Goossens H, Versporten A, Costa SF; Brazilian Global-PPS Working Group. Global point prevalence survey of antimicrobial consumption in Brazilian hospitals. J Hosp

Infect. 2020;104(2):165-171.

17. Nunes PHC, Moreira JPL, Thompson AF, Machado TLDS, Cerbino-Neto J, Bozza FA. Antibiotic Consumption and Deviation of Prescribed Daily Dose From the Defined Daily Dose in Critical Care Patients: A Point-Prevalence Study. Front Pharmacol. 2022;16;13:913568.
18. Al Matar M, Enani M, Binsaleh G, Roushdy H, Alokaili D, Al Bannai A, et al. Point prevalence survey of antibiotic use in 26 Saudi hospitals in 2016. J Infect Public Health. 2019;12(1):77-82.
19. Frenette C, Sperlea D, German GJ, Afra K, Boswell J, Chang S, et al. The 2017 global point prevalence survey of antimicrobial consumption and resistance in Canadian hospitals. Antimicrob Resist Infect Control. 2020;11;9(1):104.
20. Sözen H, Gönen I, Sözen A, Kutlucan A, Kalemci S, Sahan M. Application of ATC/DDD methodology to evaluate of antibiotic use in a general hospital in Turkey. Ann Clin Microbiol Antimicrob. 2013;3;12:23.
21. Guclu E, Ogutlu A, Karabay O, Demirdal T, Erayman I, Hosoglu S, et al. Antibiotic consumption in Turkish hospitals; a multi-centre point prevalence study. J Chemother. 2017;29(1):19-24.
22. Karabay O, Ince N, Aypak A, Guclu E, Bodur H; Group EKMUD Antibiotic Study. Antibiotic usage in hospitalized patients: a one-day point prevalence study. J Chemother. 2020;32(4):188-192.
23. Rameshkumar MR, Arunagirinathan N. Drug-resistant bacterial infections in HIV patients. Advances in HIV and AIDS Control, 2018;83.
24. World Health Organization [Internet]. WHO Collaborating Centre for Drug Statistics Methodology, Guidelines for ATC classification and DDD assignment, 2023. Oslo, 2022. Accesed January 16 2024 Available from: https://www.whocc.no/filearchive/publications/2023_guidelines_web.pdf
25. Bitterman R, Hussein K, Leibovici L, Carmeli Y, Paul M. Systematic review of antibiotic consumption in acute care hospitals. Clin Microbiol Infect. 2016;22(6):561.e7-561.e19.
26. Vu TVD, Do TTN, Rydell U, Nilsson LE, Olson L, Larsson M, et al. Antimicrobial susceptibility testing and antibiotic consumption results from 16 hospitals in Viet Nam: The VINARES project 2012-2013. J Glob Antimicrob Resist. 2019;18:269-278.
27. Kim B, Hwang H, Kim J, Lee MJ, Pai H. Ten-year trends in antibiotic usage at a tertiary care hospital in Korea, 2004 to 2013. Korean J Intern Med. 2020;35(3):703-713.
28. Antimicrobial Use and Resistance in Australia (AURA) 2021. Fourth Australian report on antimicrobial use and resistance in human health. Accesed 21 January 2024 Available from: https://www.safetyandquality.gov.au/sites/default/files/2021-09/aura_2021_-_report_-_final_accessible_pdf_-_for_web_publication.pdf
29. Dumartin C, L'Hériteau F, Péfau M, Bertrand X, Jarno P, Boussat S, et al. Antibiotic use in 530 French hospitals: results from a surveillance network at hospital and ward levels in 2007. J Antimicrob Chemother. 2010;65(9):2028-36.
30. Sekoni KF, Oreagba IA, Oladoja FA. Antibiotic utilization study in a teaching hospital in Nigeria. JAC Antimicrob Resist. 2022;5;4(5):dlac093.
31. Romo-Castillo HF, Pazin-Filho A. Towards implementing an antibiotic stewardship programme (ASP) in Ecuador: evaluating antibiotic consumption and the impact of an ASP in a tertiary hospital according to World Health Organization (WHO) recommendations. J Glob Antimicrob Resist. 2022;29:462-467.
32. Borg MA, Zarb P, Ferech M, Goossens H; ARMed Project Group. Antibiotic consumption in southern and eastern Mediterranean hospitals: results from the ARMed project. J Antimicrob Chemother. 2008;62(4):830-6.
33. Trejnowska E, Deptuła A, Tarczyńska-Słomian M, Knapik P, Jankowski M, Misiewska-Kaczur A, et al. Surveillance of Antibiotic Prescribing in Intensive Care Units in Poland. Can J Infect Dis Med Microbiol. 2018;28;2018:5670238.
34. Murila BL, Nyamu DG, Kinuthia RN, Njogu PM. Rational use of antibiotics and covariates of clinical outcomes in patients admitted to intensive care units of a tertiary hospital in Kenya. Hosp Pract (1995). 2022;50(2):151-158.
35. Anand Paramadhas BD, Tiroyakgosi C, Mpinda-Joseph P, Morokotso M, Matome M, Sinkala F, et al. Point prevalence study of antimicrobial use among hospitals across Botswana; findings and implications. Expert Rev Anti Infect Ther. 2019;17(7):535-546.

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ORIGINAL ARTICLE / ÖZGÜN MAKALE

The Impact of Genetic Variants of Angiotensin-converting enzymes (ACE1, ACE2) On the Severity of COVID-19 Disease

Anjiyotensin Dönüştürücü Enzimlerin (ACE1, ACE2) Genetik Varyantlarının COVID-19 Hastalığının Şiddeti Üzerine Etkisi

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Abstract

Objective: This study aimed to explore whether there is a potential link between ACE1 I/D and ACE2 rs2285666 polymorphisms and COVID-19 severity.
Materials and Methods: A prospective observational study was conducted involving 200 patients who were diagnosed with COVID-19 through polymerase chain reaction testing. Demographic and clinical data were collected, and genetic analyses of ACE1 I/D and ACE2 rs2285666 genes were carried out using next-generation sequencing. Patients were classified into three groups based on disease severity: mild, moderate, and severe.
Results: The average age of participants was 52 (±27) years, with 116 (58%) being male. Among them, 120 (60%) had at least one chronic illness, and one-fourth were smokers. Fifty-two (26%) patients with severe symptoms required intensive care, and 19 (9.5%) of these individuals unfortunately passed away. Meanwhile, the remaining 74% with mild or moderate symptoms were discharged after recovering. No statistically significant association was found between ACE1 I/D and ACE2 rs2285666 polymorphisms and COVID-19 severity or mortality.
Conclusion: The results indicate that ACE1 I/D and ACE2 rs2285666 polymorphisms do not significantly impact the severity of COVID-19. Further studies including diverse ethnic groups and examining other polymorphisms are needed to provide a more comprehensive understanding of these genetic influences.
Keywords: ACE, clinical severity, COVID-19, genetic polymorphism

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Öz

Amaç: Bu çalışmada, ACE1 I/D ve ACE2 rs2285666 polimorfizmleri ile COVID-19 şiddeti arasındaki ilişkinin araştırılması amaçlandı.
Yöntem: Bu çalışma, Polimeraz zincir reaksiyonu yöntemi ile COVID-19 tanısı konulan 200 hasta üzerinde prospektif, gözlemsel olarak gerçekleştirildi. Çalışmaya alınan olguların demografik ve klinik verileri kaydedilerek, ACE1 I/D ve ACE2 rs2285666 genlerinin genetik analizleri, yeni nesil dizi yöntemi kullanılarak gerçekleştirildi. Vakalar, hastalığın şiddetine göre hafif, orta ve ağır olarak üç gruba ayrıldı.
Bulgular: Çalışmaya alınan olguların ortalama yaşı 52 (±27) yıl olup, 116'sı (%58) erkekti. Olguların 120'sinde (%60) en az bir kronik hastalık mevcutken, dörtte birinde düzenli sigara kullanımı vardı. Ağır klinik bulguları olan 52 hasta (%26) yoğun bakım servisinde tedavi edilirken ve bunların 19'u (%9,5) hayatını kaybetti. Kalan %74'lük hafif veya orta şiddette klinik semptomları olan olguların tümü iyileşerek taburcu edildi. Çalışmada ACE1 I/D ve ACE2 rs2285666 gen polimorfizmleri ile COVID-19 şiddeti veya ölüm oranı arasında istatistiksel olarak anlamlı bir ilişki bulunamadı.
Sonuç: Bulgular, ACE1 I/D ve ACE2 rs2285666 polimorfizmlerinin COVID-19 şiddetini etkilemediğini göstermektedir. Bu ilişkinin daha kapsamlı bir şekilde anlaşılabilmesi için, farklı etnik grupları içeren ve farklı polimorfizmleri araştıran daha fazla çalışmaya ihtiyaç vardır.
Anahtar Kelimeler: ACE, klinik ağırlık, COVID-19, genetik polimorfizm

INTRODUCTION

The COVID-19 pandemic, which began in Wuhan, China, has led to a global outbreak, causing millions of infections and deaths worldwide, including in our country (1). While some individuals experience COVID-19 without symptoms, others may develop severe pneumonia, multiple organ failure, or even succumb to the disease (2). The notable variation in disease progression among infected individuals has sparked extensive research to better understand the pathogenesis, identify high-risk populations, and develop effective treatments (3). While the World Health Organization's guidelines and other sources highlight that individuals with chronic conditions and the elderly are at increased risk for COVID-19, severe cases in previously healthy individuals without apparent risk factors suggest that genetic differences may also play a role (4).

The renin-angiotensin system (RAS) significantly influences cardiovascular, respiratory, and renal systems, and it plays a key role in COVID-19 pathogenesis. SARS-CoV-2, the virus responsible for COVID-19, binds to angiotensin-converting enzyme 2 (ACE2) on cell membranes of nasopharyngeal mucosa and alveolar pneumocytes using its spike (S) protein (5). Therefore, ACE2 gene expression may influence susceptibility to SARS-CoV-2 infection (6). Moreover, the balance between angiotensin-converting enzyme 1 (ACE1) and ACE2 activity is known to impact the pathogenesis of respiratory diseases and may similarly affect COVID-19 severity (7). Limited prior studies have reported associations between ACE1 and ACE2 gene variants and disease severity, though some were based on small

sample sizes, low statistical significance, or hypothetical data (8-11). Further well-controlled clinical studies with larger patient groups are needed to deepen our understanding of COVID-19 pathogenesis and identify high-risk groups.

This study aims to investigate the role of ACE1 insertion/deletion (I/D), ACE1 deletion/deletion (D/D) gene variants, and ACE2 polymorphisms in the progression of COVID-19.

MATERIALS AND METHODS

Study Design and Population

This prospective observational study was conducted at Ege University School of Medicine, one of the largest tertiary care hospitals in Türkiye. Our study included 200 patients diagnosed with COVID-19 via polymerase chain reaction (PCR) testing. Patient demographic and clinical characteristics, chronic illness status, smoking habits, treatment settings (outpatient/inpatient/intensive care unit), and outcomes were recorded in case report forms.

Ethics approval was obtained from the Scientific Research Ethics Committee of Ege University School of Medicine. To ensure confidentiality, no patient-identifiable data was collected. The study procedures complied with the ethical standards outlined in the 1964 Helsinki Declaration, as revised in 2008, and followed national regulations.

Patients were categorized into three groups based on disease severity: "mild," "moderate," and "severe." Patients without signs of viral pneumonia or hypoxia, but with other symptoms or who were asymptomatic, were classified as "mild." Those with clinical symptoms of pneumonia not requiring hospitalization were managed at home or outpatient settings and categorized as "moderate." Patients with severe pneumonia, ARDS, sepsis, septic shock, or requiring intensive care were classified as "severe."

Chronic conditions considered included hypertension, diabetes, coronary artery disease, heart failure, COPD, malignancy, chronic kidney failure, liver failure, immunodeficiency, and conditions requiring immunosuppressive drugs.

Genetic analysis

Peripheral blood samples were collected in EDTA tubes, and genomic DNA was extracted from peripheral blood leukocytes using the QIAamp DNA Blood Kit (Qiagen, Germany). Extracted DNA was quantified and stored at -80°C until further use. Sequencing of targeted regions was conducted to assess the specified polymorphisms.

Genotyping for the ACE1 I/D polymorphism was performed using PCR with custom-designed primers. The amplicons generated were 192 base pairs for the ACE I allele and 490 base pairs for the ACE D allele, and gel electrophoresis was used to verify amplified PCR products. Bands of 192 base pairs indicated ACE1 I/I, 490 base pairs indicated ACE1 D/D, and both bands indicated ACE1 I/D.

Genotyping for the ACE2 rs2285666 polymorphism was also performed by PCR, and the amplified products were analyzed using gel electrophoresis. After successful amplification, samples were sequenced on the Illumina Miniseq platform.

Sequencing data were analyzed using a bioinformatics pipeline, and allelic status was visualized on the Integrative Genomics Viewer (IGV) software. Allele frequencies and genotypes (homozygous or heterozygous) were recorded.

Data Analysis

Statistical analyses were conducted to assess differences in allele frequencies, homozygous/heterozygous status, and under both recessive and dominant genetic models. Categorical variables were

compared using the chi-square test, while continuous variables were analyzed using one-way ANOVA. A significance threshold of $p < 0.05$ was set. All analyses were performed using SPSS software (version 26.0).

RESULTS

Among the study participants, 116 (58%) were male, and 84 (42%) were female, with an average age of 52 (±27) years. Sixty-four participants (32%) were children under 18. One hundred and twenty patients (60%) had at least one chronic illness, while 48 (24%) were regular smokers. About 75% of those with mild/moderate symptoms recovered without complications. However, 52 patients (26%) with severe symptoms required intensive care, and 19 (9.5%) did not survive.

Severely affected patients were generally older, had more chronic conditions, and were more likely to smoke compared to those with milder symptoms ($p < 0.001$, $p = 0.004$, and $p < 0.001$, respectively) (Table 1).

Table 1. Associations between disease severity and demographic and clinical characteristics of the patients

Cases Characteristics	Mild/Moderate Cases	Severe Cases		
N (%)	N=148 (74)	N=52 (26)	OR (95% CI)	P-value
Age, years (mean, ± SD)	38 ± 27	64 ± 20	-	**<0.001**
Gender, (n, %)				
Female	68(81)	16(19)		
Male	80(69)	36(31)	1.93 (0.98-3.74)	0.056
Comorbidities ( ) no.(%)	80 (54)	40 (76)	2.22 (1.24-3.96)	**0.004**
Smoking ( ) no (%)	17 (11)	31 (60)	4.67 (2.98-7.32)	**<0.001**

CI = Confidence interval; OR = Odds ratio

Deceased patients were more often male, older, had chronic illnesses, and smoked more frequently than survivors ($p = 0.001$, $p < 0.001$, $p = 0.004$, and $p < 0.001$, respectively) (Table 2).

Table 2. Associations between mortality and demographic and clinical characteristics of the patients

Cases Characteristics	Survivors	Died		
N (%)	N=181 (90.5)	N=19 (9.5)	OR (95% CI)	P-value
Age, years (mean, ± SD)	43 ± 27	69 ± 12	-	**<0.001**
Gender, (n, %)				
Female	83 (99)	1 (1)		
Male	98 (84)	18 (16)	15.24 (1.99-116.63)	**0.001**
Comorbidities ( ) no (%)	104 (57)	16 (84)	3.55 (1.07-11.80)	**0.024**
Smoking ( ) no (%)	17 (19)	14 (74)	8.86 (3.36-23.34)	**<0.001**

CI = Confidence interval; OR = Odds ratio

No statistically significant associations were observed between the ACE1 I/D or ACE2 rs2285666 polymorphisms and disease severity or mortality (Tables 3-6).

Table 3. The relationship between ACE1 I/D gene polymorphism and disease severity

Genetic model N (%)	Mild/Moderate Cases N=148 (74)	Severe Cases N=52 (26)	OR (95% CI)	P-value
DI & II	55 (72.4) / 40 (83.3)	21 (27.6) / 8 (16.7)	1.90 (0.76-4.74)	0.160
DD & II	53 (69.7) / 40 (83.3)	23 (30.3) / 8 (16.7)	0.46 (0.18-1.13)	0.089
DD & DI	53 (69.7) / 55 (72.4)	23 (30.3) / 21 (27.6)	0.88 (0.43-1.77)	0.721
DD + DI & II (Dominant model)	108 (71.1) / 40 (83.3)	44 (28.9) / 8 (16.7)	0.49 (0.21-1.13)	0.091
II + DI & DD (Recessive model)	95 (76.6) / 53 (69.7)	29 (23.4) / 23 (30.3)	0.70 (0.37-1.33)	0.282

ACE= Angiotensin-converting enzymes; CI = Confidence interval; OR = Odds ratio

Table 4. The relationship between ACE1 I/D gene polymorphism and mortality

Genetic model N (%)	Survivors N=181 (90.5)	Died N=19(9.5)	OR (95% CI)	P-value
DI & II	71 (93.4) / 44 (91.7)	5 (6.6) / 4 (8.3)	0.77 (0.19-3.04)	0.714
DD & II	66 (86.8) / 44 (91.7)	10 (13.2) / 4 (8.3)	0.60 (0.17-2.03)	0.563
DD & DI	66 (86.8) / 71 (93.4)	10 (13.2) / 5 (6.6)	0.46 (0.15-1.43)	0.174
DD + DI & II (Dominant model)	137 (90.1) / 44 (91.7)	15 (9.9) / 4 (8.3)	0.83 (0.26-2.63)	0.503
II + DI & DD (Recessive model)	115 (92.7) / 66 (86.8)	9 (8.3) / 10 (13.2)	0.51 (0.20-1.33)	0.167

ACE= Angiotensin-converting enzymes; CI = Confidence interval; OR = Odds ratio

Table 5. The relationship between ACE2 rs2285666 gene polymorphism and disease severity

Genetic model N (%)	Mild/Moderate Cases N=148 (74)	Severe Cases N=52 (26)	OR (95% CI)	P-value
CT & TT	27 (81.8) / 19 (67.9)	6 (18.2) / 9 (32.1)	0.46 (0.14-1.53)	0.207
CC & TT	102 (73.4) / 19 (67.9)	37 (26.6) / 9 (32.1)	1.30 (0.54-3.14)	0.551
CC & CT	102 (73.4) / 27 (81.8)	37 (26.6) / 6 (18.2)	0.61 (0.23-1.60)	0.314
CC + CT & TT (Dominant model)	129 (75) / 19 (67.9)	43 (25) / 9 (32.1)	1.42 (0.59-3.37)	0.424
TT + CT & CC (Recessive model)	46 (75.4) / 102 (73.4)	15 (24.6) / 37 (26.6)	0.89 (0.44-1.79)	0.763

ACE= Angiotensin-converting enzymes; CI = Confidence interval; OR = Odds ratio

Table 6. The relationship between ACE2 rs2285666 gene polymorphism and mortality

Genetic model N (%)	Survivors N=181 (90.5)	Died N=19(9.5)	OR (95% CI)	P-value
CT & TT	32 (97) / 26 (92.9)	1 (3) / 2 (7.1)	0.40 (0.03-4.73)	0.438
CC & TT	123 (88.5) / 26 (92.9)	16 (11.5) / 2 (7.1)	0.59 (0.12-2.73)	0.386
CC & CT	123 (88.5) / 32 (97)	16 (11.5) / 1 (3)	0.24 (0.03-1.88)	0.121
CC + CT & TT (Dominant model)	155 (71.1) / 26 (92.9)	17 (90.1) / 2 (7.1)	0.70 (0.5-3.21)	0.484
TT + CT & CC (Recessive model)	58 (76.6) / 123 (88.5)	3 (23.4) / 16 (11.5)	0.39 (0.11-1.41)	0.193

ACE= Angiotensin-converting enzymes; CI = Confidence interval; OR = Odds ratio

DISCUSSION

This study analyzed the association between ACE1 I/D and ACE2 rs2285666 polymorphisms and COVID-19 severity in a cohort of 200 patients diagnosed across all age groups. Our findings indicated that neither the ACE1 I/D nor the ACE2 rs2285666 polymorphisms significantly influenced the clinical course of COVID-19. However, disease severity was more pronounced in male, elderly, chronically ill, and smoking patients.

Previous studies, such as those by Hubacek et al. in the Czech Republic, Aladağ et al. in Turkey, and Verma et al. in India, reported various associations between ACE1 gene polymorphisms and symptomatic or severe COVID-19, although results were not always consistent and did not conclusively link these variants to hospitalization or mortality outcomes (12-14).

Delanghe et al. conducted a study to compare the prevalence and mortality rates of COVID-19 across several European countries with the geographical distribution of the I/D polymorphism in the ACE gene (4). Unlike other studies, the authors found a negative correlation between the frequency of the D allele of the ACE I/D polymorphism and COVID-19 prevalence and mortality in 33 countries. Its results conflicted with data from East Asian populations[15,16]. The study suggested an inverse relationship between the frequency of the ACE D allele and COVID-19 prevalence. Given that the D allele frequency is lower in Asian populations compared to European populations, a higher prevalence and mortality rate of COVID-19 would be expected in Asia (14-16).

In Poland Sienko et al. have determined that patients with ACE2 rs2285666 AA, ACE2 rs2074192 TT, and ACE2 rs4646174 GG gene variants had a more severe course of COVID-19 (16). However, other studies conducted in Turkey and Germany did not find associations between ACE2 rs2285666 polymorphisms and disease progression (17,18). The observed discrepancies could be attributed to the different distribution of alleles across populations.

Since the onset of the COVID-19 outbreak, numerous researchers have examined the epidemiological characteristics of individuals who were more frequently affected or experienced a more severe form of the disease (18-22). Consistent with our findings, most of them have indicated that those infected were more likely to have chronic illnesses, be male, older in age, and/or be smokers.

There are some limitations to this study, including being conducted at a single center, which limits generalizability. Furthermore, variation in clinical assessment may have influenced severity classification. The results may also be influenced by the fact that many COVID-19 cases recover without hospitalization.

CONCLUSION

This study, the largest of its kind in Turkey, explored the impact of ACE1 I/D and ACE2 rs2285666 polymorphisms on COVID-19 severity across all age groups. Our study does not provide evidence supporting the hypothesis that ACE1 I/D and ACE2 rs2285666 polymorphisms are associated with the severity of COVID-19. Additionally, our findings indicate that age, gender, chronic illnesses, and smoking play significant roles in determining the clinical severity of COVID-19. Genetic factors may still contribute to COVID-19 severity, but underlying health conditions seem to play a more decisive role. Further research with larger, ethnically diverse cohorts is required to clarify the role of genetic variations in COVID-19 outcomes.

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REFERENCES

1. World Health Organization. Coronavirus Disease (COVID-19) Dashboard. Geneva, Italy: WHO; 2022. Available from: https://data.who.int/dashboards/covid19/cases
2. World Health Organization. Clinical management of COVID-19: living guideline. Geneva, Italy: WHO; 2023. Available from: https://www.who.int/publications/i/item/WHO-2019-nCoV-clinical-2023.2
3. Wang B, Li R, Lu Z, Huang Y. Does comorbidity increase the risk of patients with COVID-19: evidence from meta-analysis. Aging. 2020;12(7):6049-57.
4. Delanghe JR, Speeckaert MM, De Buyzere ML. COVID-19 infections are also affected by human ACE1 D/I polymorphism. Clinical Chemistry and Laboratory Medicine. 2020;58(7):1125-6.
5. Marshall RP, Webb S, Bellingan GJ, Montgomery HE, Chaudhari B, McAnulty RJ, et al. Angiotensin converting enzyme insertion/deletion polymorphism is associated with susceptibility and outcome in acute respiratory distress sendrome. American Journal of Respiratory and Critical Care Medicine. 2002;166(5):646-50.
6. Gomez J, Albaiceta GM, Garcia-Clamente M, López-Larrea C, Amado-Rodríguez L, Lopez-Alonso I, et al. Angiotensin-converting enzymes (ACE, ACE2) gene variants and COVID-19 outcome. Gene. 2020;762(1):145 102.
7. Annunziata A, Coppola A, Di Spirito V, Cauteruccio R, Marotta A, Di Micco P, Fiorentino G. The Angiotensin Converting Enzyme Deletion/Deletion Genotype Is a Risk Factor for Severe COVID-19: Implication and Utility for Patients Admitted to Emergency Department. Medicina. (Kaunas) 2021;57(8):844.
8. Iwai M, Horiuchi M. Devil and angel in the renin-angiotensin system: ACE-angiotensin 2-AT1 receptor axis vs. ACE2-angiotensin-(1-7)-Mas receptor axis. Hypertension Research. 2009;32(7):533-6.
9. Zheng H, Cao J.J. Angiotensin-Converting Enzyme Gene Polymorphizm and Severe Lung Injury in Patients with Coronavirus Disease 2019. The American Journal of Pathology. 2020;190(10):2013-7.
10. Aziz MA, Islam MS. Association of ACE1 I/D rs1799752 and ACE2 rs2285666 polymorphisms with the infection and severity of COVID-19: A meta-analysis. Molecular Genetics & Genomic Medicine. 2022;10(11):e2063.
11. Liu Y, Yang Y, Zhang C, Huang F, Wang F, Yuan J, et al. Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. Science China Life Sciences. 2020;63(3):364-74.

12. Hubacek JA, Dusek L, Majek O, Adamek V, Cervinkova T, Dlouha D, Adamkova V. ACE I/D polymorphism in Czech first-wave SARS-CoV-2-positive survivors. Clinica Chimica Acta. 2021;519(3):206-9.
13. Aladag E, Tas Z, Ozdemir BS, Akbaba TH, Akpınar GM, Goker H, et al. Human Ace D/I Polymorphism Could Affect the Clinicobiological Course of COVID-19. Journal of the Renin-Angiotensin-Aldosterone System. 2021;5509280.
14. Verma S, Abbas M, Verma S, Khan FH, Raza ST, Siddiqi Z, et al. Impact of I/D polymorphism of angiotensin-converting enzyme 1 (ACE1) gene on the severity of COVID-19 patients. Infection, Genetics and Evolution. 2021;91:104801.
15. Saadat M. No significant correlation between ACE Ins/Del genetic polymorphism and COVID-19 infection. Clin Chem Lab Med. 2020;58:1127-1128.
16. Pati A, Mahto H, Padhi S, Panda AK. ACE deletion allele is associated with susceptibility to SARS-CoV-2 infection and mortality rate: an epidemiological study in the Asian population. Clin Chim Acta. 2020;510(3):455-458
17. Sienko J, Marczak I, Kotowski M, Bogacz A, Tejchman K, Sienko M, Kotfis K. Association of ACE2 Gene Variants with the Severity of COVID-19 Disease-A Prospective Observational Study. International Journal of Environmental Research and Public Health. 2022;19(2):12622.
18. Çelik, SK, Genç GC, Pişkin N, Açikgöz B, Altinsoy B, Kurucu İşsiz B, Dursun A. Polymorphisms of ACE (I/D) and ACE2 receptor gene (Rs2106809, Rs2285666) are not related to the clinical course of COVID-19: A case study. Journal of Medical Virology. 2021;93(10):5947-52.
19. Möhlendick B, Schönfelder K, Breuckmann K, Elsner C, Babel N, Balfanz P, et al. ACE2 polymorphism and susceptibility for SARS-CoV-2 infection and severity of COVID-19. Pharmacogenetics and Genomics. 2021;31(8):165-71.
20. Shekerdemian LS, Mahmood NR, Wolfe KK, Riggs BJ, Ross CE, McKiernan CA. Characteristics and Outcomes of Children With Coronavirus Disease 2019 (COVID-19) Infection Admitted to US and Canadian Pediatric Intensive Care Units. JAMA Pediatr 2020;174(9):868-473.
21. CDC COVID-19 Response Team. Coronavirus disease 2019 in children — United States, February 12–April 2, 2020. MMWR Morb Mortal Wkly Rep. 2020;69(14):422-426.
22. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395(20):497-506.

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ORIGINAL ARTICLE / ÖZGÜN MAKALE

Parkinson Hastalığında SİBO (İnce Bağırsak Bakteri Aşırı Çoğalması): WOS örneği ile Bibliyometrik İnceleme

SIBO (Small Intestinal Bacterial Overgrowth) in Parkinson's Disease: Bibliometric Review with WOS Example

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Öz

Amaç: Bu çalışmada Parkinson Hastalığında (PH) ince bağırsak bakteri aşırı çoğalması (SİBO) konusunda 2024 öncesi yayınlanan makalelerin niteliksel özelliklerinin araştırılması ve ileride yapılacak çalışmalar için araştırmacılara yol gösterilmesi amaçlanmıştır.
Gereç ve Yöntemler: Web of Science veri tabanında arama kriterlerine "Parkinson Disease" ve "Small İntestinal Bacterial Overgrowth" anahtar kelimeleri girilerek tespit edilen 41 makale önceden belirlenmiş olan ölçütler etrafında değerlendirilmiştir. Bu çalışmada verilerin analizinde içerik analizi tekniğinden yararlanılarak bir durum çalışma örneği ortaya konmuştur. Yayınların yılları ve tipleri, yayın yapan ülkeler ve merkezler, yayınların yer aldığı dergiler, yayın yapan araştırmacılar, yayınların atıf alma sayıları ve en fazla atıf alan yayınların incelemeleri yapılmıştır.
Bulgular: WOS veri tabanında ulaşılan 41 makaleden 26 tanesinin incelenmek istenen konuyla ilişkisi tespit edilmiştir. Bu konuda yapılan ilk yayın 2005 yılına aittir ve yayınların çoğunluğunu makaleler oluşturmaktadır. En çok yayın yapan bilim dalları klinik nöroloji ve gastroenteroloji olup en fazla yayının bulunduğu dergiler Parkinsonism and Related Disorders, Movevemet Disorders ve Gastroenterology'dir. Kanada, Çin ve USA en fazla yayının üretildiği ülkeler olup en fazla yayın yapan merkezler sırasıyla Universty Healthy Network Toronto ve Universty of Toronto'dur. En çok atıf alan yayın olan "Gastrointestinal dysfunction in Parkinson's disease" 2015 yılında Fasona ve arkadaşları tarafından yapılmış olup Lancet Neurology 'de yayınlanmıştır.
Sonuç: Parkinson hastalığı ve SİBO ilişkisini inceleyen çalışma sayısı az olmakla birlikte son yıllarda dünya literatüründe konuya olan ilginin arttığı tespit edilmiştir. Bu konuda ülkemizdeki merkezlere ait yayın bulunmamakla birlikte ülkemizde yayınlanan dergilerde de herhangi bir yayına rastlanmaması dikkat çekmektedir. Bu konudaki araştırmaların Parkinson Hastalığının etyopatogenezine ışık tutacak ve hastaların klinik bulgularında iyileşmeye katkıda bulunabilecek sonuçlar elde etme potansiyelinden dolayı özellikle genç araştırmacılar tarafından tercih edilebilecek için bir çalışma alanı olacağı düşüncesindeyiz.
Anahtar Kelimeler: Parkinson Hastalığı, ince bağırsak bakteri aşırı çoğalması, SİBO, bibliyometrik analiz

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Abstract

Aim: This study aims to investigate the qualitative characteristics of articles published before 2024 on small intestinal bacterial overgrowth (SIBO) in Parkinson's Disease (PD) and to guide researchers for future studies.
Materials and Methods: The 41 articles identified by entering the keywords "Parkinson Disease" and "Small Intestinal Bacterial Overgrowth" into the search criteria in the Web of Science database were evaluated around the previously determined criteria. In this study, a case study example was presented by using the content analysis technique in the analysis of the data. The years and types of publications, the countries and centers that published, the journals in which the publications were published, the researchers who published, the number of citations of the publications and the most cited publications were examined.
Results: When the 41 articles accessed in the WOS database were examined, it was determined that 26 of them were related to the subject to be examined. The first publication on this subject was from 2005 and the majority of the documents were articles. The most published branches are clinical neurology and gastroenterology, and the journals with the most publications are Parkinsonism and Related Disorders, Movevemet Disorders, and Gastroenterology. Canada, China, and the USA are the countries with the most publications, and the centers with the most publications are University Healthy Network Toronto and University of Toronto, respectively. The most cited publication, "Gastrointestinal dysfunction in Parkinson's disease", was made by Fasona and colleagues in 2015 and published in Lancet Neurology.
Conclusion: Although the number of studies examining the relationship between Parkinson's disease and SIBO is low, it has been determined that interest in the subject has increased in the world literature in recent years. While there are no publications from centers in our country on this subject, it is also noteworthy that no publications are found in journals published in our country. We believe that research on this subject will be a field of study that can be preferred especially by young researchers due to its potential to shed light on the etiopathogenesis of Parkinson's disease and to obtain results that can contribute to the improvement in the clinical findings of patients.
Keywords: Parkinson's Disease, small intestinal bacterial overgrowth, SIBO, bibliometric analysis

GİRİŞ

Parkinson Hastalığı nörolojik hastalıkların arasında prevalansı en fazla artan, Alzheimer'den sonra 2. en sık görülen nörodejeneratif hastalıktır (1,2). PH erkeklerde daha sık görülen, asimetrik, tremor, hareketlerde yavaşlama, maske yüz, öne doğru eğilimli postür ile ufak adımlarla yürüme şeklinde kendini gösteren progresif bir hastalıktır (). Sayılan belirtiler dışında tanı alınmadan önce kabızlık, omuz ağrısı, otonomik bozukluklar veya uyku bozuklukları gözlenebilir. Dünya geleninde 6 milyonun üzerinde sayıda insanı etkilediği bildirilmektedir. Hastalığın bilinen bir tedavisi yoktur (1,2,3). Patolojik bakış açısından, PD esas olarak alfa-sinüklein fosforilasyonu ve yanlış katlanması ile karakterize edilir ve bu da çözünmeyen agregatların (yani Lewy cisimcikleri ve nörit) intranöronal birikimi ve ardından nörodejenerasyonla birlikte nöroinflamatuar değişikliklerle sonuçlanır. PH'nin etyolojisinde genetik ve çevresel etkenlerin neden olduğu karmaşık bir süreçten söz edilir. Barsak mikrobiyotasının etyopatojenezde önemli bir rol oynadığı son yıllarda yapılan çalışmalarla gösterilmiştir (3).

İnce bağırsak bakteriyel aşırı çoğalması (SIBO), ince bağırsağın genel olarak kalın bağırsakta bulunan bakteriler tarafından aşırı kolonize edildiği bir bağırsak disbiyozudur (4). Esas olarak, azalmış gastrointestinal motilite/artmış gastrointestinal geçiş süresi gibi yatkınlık yaratan durumlara sahip bireylerde görülür (4,5). Çoğunlukla aşırı çoğalan bağırsak mikrobiyotası tarafından üretilen gazların veya bağırsak iltihabının neden olduğu bağırsak gerginliğiyle ilişkili,

spesifik olmayan semptomlara yol açabilir, ancak mutlaka semptomatik değildir ve bir hastalık değildir. SIBO ile ilişkili semptomlar tipik olarak bir veya birkaç antibiyotik küründen sonra kaybolur, ancak yatkınlık yaratan durum devam ederse tekrarlar (4,5,6). SIBO'nun potansiyel immünomodülatör/proinflamatuar etkileri, bağırsak bariyer geçirgenliği üzerindeki etkisi ve ince bağırsakta üretilen mikrobiyal gazlar ve diğer mikrobiyal ürünlerin seviyeleri üzerindeki potansiyel etkileri göz önüne alındığında, SIBO'nun nörodejeneratif bozuklukların etyopatogenezinde yer alması makul görünmektedir. PH'da SİBO normal popülasyona göre çok daha sık olarak bildirilmektedir (3,7,8,9).

Literatürde yayınlanan ve güncel konuları işleyen araştırmaların değerlendirilerek alana ait verilerin gözden geçirilip özet verilerin sunulmasını sağlayan bibliometrik incelemeler ile gelecek çalışmaların planlanmasına yardımcı olunmaya çalışılır. Bibliometrik analizler ile seçilmiş olan konularla ilgili çalışmalar özetlenir. Bu çalışmada PH prevelansının artığının izlendiği ve özellikle mikrobiyatanın hastalık patogenezinde önemli rol oynadığı gösterildiği bu dönemde güncel bir konu olan SİBO ile ilgili literatürü gözden geçirmek ve bilim dünyasının ilgisini belirlemek ve ayrıca geleceğe ışık tutmak amacıyla bu çalışma planlanmıştır. Bu çalışma ile, PH ve SİBO konusunda yayınlanmış olan makalelerin, yayın tipinin, yayınlandıkları yılların, en fazla yayın yapan ülkeler ve merkezler, ilgili araştırmacılar ve alandaki en çok atıf alan yayınların dokümente edilmesine, içeriklerinin incelenmesine ve genç araştırmacıların dikkatinin çekilmesine çalışılmıştır.

GEREÇ VE YÖNTEM

Araştırma Modeli

Bu makale, nitel araştırma modeline göre yürütülmüş olan bir durum çalışması olarak PH ve SİBO konusunda yayınlanmış makalelerin farklı değişkenler açısından irdelenmesi içermektedir. Söz konusu konuda ilgili makaleleri inceleyerek, PH ve SİBO alanyazın ilişkin kapsamlı bir değerlendirme yapılmaya çalışılmıştır.

WOS veri tabanında yer alan PH ve SİBO konusundaki yayınlar bu araştırmanın evrenini oluşmaktadır. Araştırmanın örneklemi ise veri tabanlarında yayınlanmış olan toplam 41 makaleden oluşmaktadır. Bu çalışmada kullanılan ölçütlerin belirlenmesinde öncelikle alanyazın yapılmış olan benzer çalışmalar incelenmiştir. Araştırmacılar tarafından alanyazın taraması sonrası hazırlanan ölçüt listesi uzman görüşüne sunulmuş, uzmanlardan gelen geribildirimlere göre liste düzenlenerek son hali verilmiştir.

Verilerin Toplanması ve Analizi

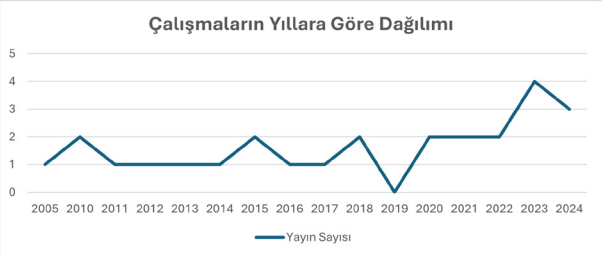
Çalışmada değerlendirilecek makale ve yazılara WOS (Web of Science) veri tabanlarından ulaşılmıştır.

BULGULAR

Yapılan taramalar sonrası toplam 41 adet yayına ulaşıldı. Tüm yayınlar incelendi ve araştırma konusu ile ilgili olmayan yayınlar elendiğinde kalan 26 yayın değerlendirmeye alındı. Çalışmaların yayınlanma yıllarına göre sıralamasına baktığımızda ilk yayının 2005 yılına ait olduğu görüşmüştür (Tablo 1) (10-32) ve özellikle 2022 yılı sonrası yayın sayısındaki artış da dikkat çekmiştir ve bu da alanın gelecekteki araştırmacılar için odak noktası olabileceğini göstermektedir (Grafik 1).

Tablo 1. Yıllara yayınların göre dağılımı

Yılı	Sayı	%
2024	3	11.5
2023	4	15.3
2022	2	7.7
2021	2	7.7
2020	2	7.7
2018	2	7.7
2017	1	3.8
2016	1	3.8
2015	2	7.7
2014	1	3.8
2013	1	3.8
2012	1	3.8
2011	1	3.8
2010	2	7.7
2005	1	3.8



Grafik 1. Çalışmaların yıllara göre dağılımı.

WOS veri tabanında elde edilen verilerden oluşan doküman tipi analizi incelendiğinde yayınların %50'si makale geri kalanların çoğunluğunu ise derleme ve kongre sunumları oluşturmaktadır (Tablo 2).

Tablo 2. Dokümanların tiplerine göre dağılımı

Yayın Tipi	Oran	%
Makale	13	50
Kongre sunumu	6	23
Derleme	6	23
Erken Baskı	1	3.8
Editöre Mektup	1	3.8

Yayınların %42'3'ü klinik nöroloji alanında olup bundan sonraki %26.9'luk dilimi gastroenteroloji alanındaki yayınlar oluşturmaktadır (Tablo3).

Tablo 3. Yayınların bilim alanlarına göre dağılımı

Yayın Alanı	Oran	%
Klinik Nöroloji	11	42.3
Gastroenteroloji	7	26.9
Nörobilim	5	19
Mikrobiyoloji	3	11.5
Biyokimya Moleküler Biyoloji	2	19.2
Deneysel Tıbbi Araştırma	2	7.7
Tıbbi Kimya	1	3.8
Multidispliner Kimya	1	3.8
Nörogörüntüleme	1	3.8
Farmakoloji	1	3.8

Bu konuda en fazla yayının yer aldığı üç dergi Parkinsonism and Related Disorders, Movevemet Disorders ve Gastroenterology'dir (Tablo4). Ülkemize ait dergilerde herhangi bir yayına rastlanmamıştır.

Tablo 4. Yayın dergileri ve sayıları.

Dergi Adı	Sayısı	%
Parkinsonism and Related Dis.	3	11.5
Movement Dis.	3	11.5
Gastroenterology	3	11.5
Gut Pathogens	2	7.7
Lancet Neurology	1	3.8
Brain İmaging and Beh.	1	3.8
Seminary in Neurology	1	3.8
World Journal of Gastroenterology	1	3.8
European Neurology	1	3.8
Advances in Clınıcal and Experimental Medicine	1	3.8
Mysytems	1	3.8
Int. Journal of Molecular Sciences	1	3.8
Acs Chemical Neuroscience	1	3.8
Medical Hypotheses	1	3.8
Journal of Parkinsons Disease	1	3.8
Journal of The Neurological Sciences	1	3.8
Journal of Neural Tramsmıssıon	1	3.8
Exper Opınıon on Pharmacoherapy	1	3.8
Digestive and Liver Disease	1	3.8

Bu konuda en fazla yayın yapan ülkeler Kanada, Çin ve USA'dir. Daha sonraki sıralamalar İtalya, Japonya ve Hollanda'ya aittir. Ülkemize ait yayın bulunmamaktadır. (Tablo5)

Tablo 5. Yayınların ülkelerine göre dağılımı

Ülke Adı	Sayı	%
Kanada	5	19.2
Çin	5	19.2
USA	5	19.2
İtalya	4	15.3
Japonya	2	7.7
Hollanda	2	7.7
Avustralya	1	3.6
İngiltere	1	3.6
Malezya	1	3.6
Polonya	1	3.6
Romanya	1	3.6
Rusya	1	3.6

Yayınların üretildiği ilk 5 merkezin isimleri ve yayın sayısı Tablo 6'de gösterilmiştir.

Tablo 6. En sık yayın üreten merkezlerin isim ve sayısı

Universty Healthy Network Toronto	5
Universty of Toronto	5
Catholic Universty of The Sacred Heart	3
IRCCS Policlinico Gemelli	3
Oregon Health Sicence Universty	2

Çalışmaların aldığı atıf sayıları incelenerek hazırlanan en fazla atıf alan 5 yayının künye bilgileri Tablo 7'de verilmiştir.

Tablo 7. En fazla atıf yapılan 5 yayının künyeleri

Başlık	Yazarlar	Yayın dergi	Yayın Yılı	Toplam Atıf	Yılda ortalama Atıf
Gastrointestinal Dysfunction in Parkinson's Disease.	Fasano, A., Visanji, N. P., Liu, L. W., Lang, A. E., & Pfeiffer, R. F.	The Lancet Neurology	2015	557	61.8
The role of small intestinal bacterial overgrowth in Parkinson's disease	Fasano, A., Bove, F., Gabrielli, M., Petracca, M., Zocco, M. A., Ragazzoni, E., Barbaro, F., Piano, C., Fortuna, S., Tortora, A., Di Giacopo, R., Campanale, M., Gigante, G., Lauritano, E. C., Navarra, P., Marconi, S., Gasbarrini, A., & Bentivoglio, A. R.	Movement disorders	2013	241	20.9
Small intestinal bacterial overgrowth in Parkinson's disease	Tan, A. H., Mahadeva, S., Thalha, A. M., Gibson, P. R., Kiew, C. K., Yeat, C. M., Ng, S. W., Ang, S. P., Chow, S. K., Tan, C. T., Yong, H. S., Marras, C., Fox, S. H., & Lim, S. Y.	Parkinsonism & related disorders.	2014	192	19.2
Prevalence of small intestinal bacterial overgrowth in Parkinson's disease.	Gabrielli, M., Bonazzi, P., Scarpellini, E., Bendia, E., Lauritano, E. C., Fasano, A., Ceravolo, M. G., Capecci, M., Rita Bentivoglio, A., Provinciali, L., Tonali, P. A., & Gasbarrini, A.	Movement disorders	2011	129	9.9
Association of Intestinal Disorders with Parkinson's Disease and Alzheimer's Disease: A Systematic Review and Meta-Analysis.	Fu, P., Gao, M., & Yung, K. K. L.	ACS chemical neuroscience,	2020	85	21.2

PH ve SİBO arasındaki bağlantıyı inceleyen yayınlardan en fazla atıf alan yayınlara baktığımızda SİBO'nun PH etyopatogenezindeki rolü hakkında bilgi vermektedir. Bunlardan en çok atıf yapılan birinci yayın olan Fasano ve arkadaşlarının yayınladıkları Parkinson hastalığında gastrointestinal disfonksiyonları irdeledikleri derlemedir (24). Bu derlemede kısaca Parkinson hastalığında gastrointestinal tutulum hakkındaki güncel bilgiler gözden geçirilmiştir. İlk olarak, gastrointestinal sistemin Parkinson hastalığının başlangıç yeri olabileceği hipotezini destekleyen giderek artan deneysel kanıtlar sunulmuş ve gastrointestinal α-sinüklein birikiminin (submandibular bezden apandisit ve kolona) tanısal bir biyobelirteç olarak potansiyel kullanımına dair kanıtları tartışılmıştır. İkinci olarak, gastrointestinal disfonksiyonun oral alım zorlukları, yutma sorunları ve kabızlık dahil olmak üzere klinik semptomlar üzerindeki etkileri tartışılışmıştır. Ayrıca SIBO ve Helicobacter pylori enfeksiyonunun Parkinson hastalığının patofizyolojik süreçlere ve antiparkinson ilaçların emilimi üzerindeki etkileri kanıtlarla tartışılmıştır.

İkinci yayında SİBO'nun Parkinson Hastalığında görülen motor semptomlara katkıda bulunup bulunmadığı araştırılmıştır (25). Toplam 33 hasta ve 30 kontrol, ince bağırsakta bakteriyel aşırı çoğalmayı ve Helicobacter pylori enfeksiyonunu tespit etmek için glikoz, laktuloz ve üre nefes testleri uygulanarak SİBO varlığı araştırılmıştır. Hastalara ayrıca gastrik boşalmayı değerlendirmek için ultrasonografi uygulandı. Hastaların klinik gidişatı ve motor komplikasyonlar Birleşik Parkinson Hastalığı Derecelendirme Ölçeği-IV ile değerlendirilmiş. SİBO tespit edilen hastalar rifaximin ile tedavi edilerek ve 1 ve 6 ay sonra klinik ve enstrümantal olarak yeniden değerlendirilmiştir. Hasta grubunda ince bağırsakta bakteriyel aşırı çoğalmanın yaygınlığı kontrol grubuna kıyasla anlamlı derecede daha yüksek (%54,5'e karşı %20 ,0; P = .01) bulunmuş ancak Helicobacter pylori enfeksiyonunun yaygınlığı ise anlamlı yüksekte olmadığı gösterilmiştir (%33,3'e karşı %26,7). Herhangi bir enfeksiyonu olmayan hastalarla karşılaştırıldığında, öngörülemeyen motor dalgalanmaların yaygınlığı her iki enfeksiyonu olan hastalarda anlamlı derecede daha yüksek olduğu tespit edilmiş (%8,3'e karşı %87,5; P = .008). İzole olarak SİBO olmayan hastalarla karşılaştırıldığında, izole SİBO olan hastaların günlük dinlenme süreleri daha uzundu ve daha fazla on-off atağı izlenmiş. Sonuç olarak SİBO'nun ortadan kaldırılması, levodopanın farmakokinetiğini etkilemeden motor dalgalanmalarda iyileşme ile sonuçlanmış fakat 6. ayda ince bağırsakta bakteriyel aşırı büyümenin tekrarlama oranı %43 olarak bulunmuştur.

En fazla atıf alan 3. çalışmada ise PH'de SIBO üzerine bugüne kadar yapılmış önemli bir klinik çalışmadır (26). Toplam 103 hastada SİBO için testler yapılmış ve hastalar PH açısından takip edilmiştir. Bu çalışmada SIBO, yakın zamanda hastalık tanısı konmuş hastalar da dahil olmak üzere hastaların dörtte birinde tespit edilmiştir. SIBO tespit edilen PH olgularında daha kötü gastrointestinal semptomlar yoktu fakat bağımsız olarak daha kötü motor fonksiyonunu izlenmiştir.

Yayınlarda kullanılan yazım dilinin tümünün İngilizce olduğu görülmüştür.

TARTIŞMA

Yaptığımız bu bibliyometrik çalışmada PH ve SİBO konusundaki çalışmalar niteliksel olarak değerlendirilmiştir. PH'de barsak disbiosis ve SİBO sık görülen ve hastalık progresyonunda rol oynayan bir tablo olarak görülmektedir. Dünya literatüründe bu konuya yönelik ilgi artmakta iken ülkemizde bu konuda yapılmış yayın yoktur. SIBO ile

PD'de daha kötü motor fonksiyonu arasında nedensel bir bağlantı olduğunu doğrulayan veriler toplanmaya devam etmektedir. Ayrıca disbosise yönelik nörodejeneratif süreçleri önlemek veya geciktirmek için potansiyel tedavi seçenekleri üzerinde çalışmalar mevcuttur.

Semptomatik SIBO'nun, predispozan faktörleri ortadan kaldırmayı amaçlayan müdahalelerle birlikte bir veya birkaç antibiyotik kürüyle tedavi edilmesi gerektiği genel olarak kabul edilmektedir (örneğin, gastrointestinal geçiş süreleri artmış kişilerde prokinetik kullanımı), ikincisi de SİBO'nun tekrarlanmasını önlemek için olmazsa olmaz bir koşuldur. Asemptomatik SIBO'nun klinik önemi bilinmemektedir ancak diğer bağırsak disbiyoz durumlarına benzer şekilde, biriken kanıtlar bunun olumsuz sağlık sonuçlarına sahip olabileceğini, örneğin genel bir proinflamatuar duruma, nörotoksisiteye, nörodejenerasyona vb. katkıda bulunabileceğini göstermektedir.

PH'de uzamış bağırsak geçiş süresi yaygın bir durumdur ve genellikle motor semptomların başlangıcından yıllar önce ortaya çıkar. Bu durumun SIBO'nun PH'da daha yüksek yaygınlığını açıkladığı kabul edilir. SIBO veya diğer bağırsak disbiyozları tarafından tetiklenen lokal inflamatuar yanıtlar bağışıklık sistemini aktive edebilir. Bağırsak bariyer geçirgenliğini bozabilir, sonuçta PH patolojisinin temel özellikleri olan alfa-sinüklein ile amiloidojenik etkilere sahip olabilecek çeşitli bakteri ürünleri arasında yakın bir etkileşime izin vererek alfa-sinüklein yanlış katlanmasını ve birikimini tetikler veya artırır. Ek olarak değiştirilmiş bir bağırsak bariyeri, nöronların nörodejenerasyona duyarlılığını artırabilecek potansiyel olarak proinflamatuar ve nörotoksik mikrobiyal ve mikrobiyal olmayan bileşikler için bağırsak-beyin eksenine erişim sağlar.

Tüm mikrobiyal ürünler arasında gazlar zarlardan en kolay şekilde geçer ve vücuttaki hücresel işlevlere müdahale edebilir. SIBO'lu kişilerde ince bağırsak mikrobiyotası tarafından üretilen gazın bileşimi (SİBO varlığı glikoz veya laktuloz ile oral karbonhidrat yüklemesinden sonra H2 ve metan içeriği açısından değerlendirilir) esas olarak konsantrasyona ve kolonize olan bakteri türlerine bağlıdır. Bu gazlar hem pozitif hem de negatif yollarla PH ilerlemesine doğrudan müdahale edebilir. H2 inflamasyonu ve oksidatif stresi azaltarak potansiyel olarak nöroprotektif etkilere sahiptir. Metan ise sırasıyla protein yanlış katlanmasını önleyerek ve yanlış katlanmış proteinlerin agregatlarını kaldırarak nöroprotektif veya yanlış katlanmış proteinlerin agregatını artırarak nörodejenerasyonu artırıcı etkilere sahiptir. SIBO'nun hem lokal olarak hem de beyin içinde proinflamatuar değişikliklere neden olabileceği ve bağırsak ve kan-beyin bariyerlerinin geçirgenliğini artırarak nöronları potansiyel olarak amiloidojenik ve nörotoksik bileşiklere maruz bırakabileceği ve PH'nin ilerlemesini hızlandırabileceği düşünüldüğünde, SIBO ile ilişkili semptomlar olmasa bile PH'li kişilerde bunun ortadan kaldırılması düşünülmelidir.

PH dünya genelinde sıklığının ve engelliğinin artığı nörodejeneratif bir hastalık olması nedeniyle öneminin sadece bireysel değil aynı şekilde toplumsal ve küresel ölçekli olduğunu düşünüyoruz. Multidisipliner yaklaşım ile hastalığın premotor semptomların öğrenilmesi ve dikkate alınması hastalığın tedavisinin daha erkenden planlanması için önemli olacaktır. Ülkemizde bu konuda yayın yapılmamış olması bilimsel alanda eksikliklerimizin olduğunu ve konuya gerekli önemi vermediğimizi göstermektedir. Uluslararası literatür incelendiğinde de PH'de bu konunun daha ayrıntılı olarak ele alındığı ve yıllara göre hızlı bir artış trendinin olduğu görülmektedir. Çalışmamamızın kısıtlılığı tek veri tabanın değerlendirilmesidir.

SONUÇ

Sonuç olarak bağırsak disbiyosis varlığında nörodejeneratif süreçlerin hızlandığı gösterilmiştir. Beslenme çeşitliliğinin sağlanması, uyku ritminin düzenlenmesi ve yeterli sürede hareket ve egzersizin mikrobiyota üzerinde olumlu derecede etki gösterdiği gözlenmiştir. Hastaların bu açıdan ayrıntılı takip ve tedavilerinin sağlanması ve bilinçlendirilmesinin klinik düzelmelerine faydalı olacağı kanaatindeyiz.

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KAYNAKLAR

1. Obeso, J. A., Stamelou, M., Goetz, C. G., Poewe, W., et al. Past, present, and future of Parkinson's disease: A special essay on the 200th Anniversary of the Shaking Palsy. Movement disorders: official journal of the Movement Disorder Society. 2017; 32(9): 1264–1310. https://doi.org/10.1002/mds.27115.
2. GBD 2016 Parkinson's Disease Collaborators (2018). Global, regional, and national burden of Parkinson's disease, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. The Lancet. Neurology. 2018; 17(11): 939–953. https://doi.org/10.1016/S1474-4422(18)30295-3.
3. Li, X., Feng, X., Jiang, Z., & Jiang, Z. (). Association of small intestinal bacterial overgrowth with Parkinson's disease: a systematic review and meta-analysis. Gut pathogens. 2021; 13(1); 25. https://doi.org/10.1186/s13099-021-00420-w.
4. Gasbarrini, A., Lauritano, E. C., Gabrielli, M., Scarpellini, E., Lupascu, A., Ojetti, V., & Gasbarrini, G. Small intestinal bacterial overgrowth: diagnosis and treatment. Digestive diseases. 2007; 25(3): 237–240. https://doi.org/10.1159/000103892.
5. Rezaie, A., Pimentel, M., & Rao, S. S. How to Test and Treat Small Intestinal Bacterial Overgrowth: an Evidence-Based Approach. *Current gastroenterology reports*, 2016; *18*(2): 8. https://doi.org/10.1007/s11894-015-0482-9.
6. Efremova, I., Maslennikov, R., Poluektova, E., Vasilieva, E., Zharikov, Y., Suslov, A., et al. Epidemiology of small intestinal bacterial overgrowth. World journal of gastroenterology. 2023; 29(22): 3400–3421. https://doi.org/10.3748/wjg.v29.i22.3400.
7. Wang, Q., Luo, Y., Ray Chaudhuri, K., Reynolds, R., Tan, E. K., & Pettersson, S. The role of gut dysbiosis in Parkinson's disease: mechanistic insights and therapeutic options. Brain: a journal of neurology. 2021; 144(9): 2571–2593. https://doi.org/10.1093/brain/awab156.
8. Zhou, S., Li, B., Deng, Y., Yi, J., Mao, G., Wang, R., et al. Meta-analysis of the relations between gut microbiota and pathogens and Parkinson's disease. Advances in clinical and experimental medicine: official organ Wroclaw Medical University. 2023; 32(6): 613–621. https://doi.org/10.17219/acem/157193.
9. Safarpour, D., Brumbach, B. H., Arena, M., Quinn, J., Diamond, S., Nutt, J. G., & Pfeiffer, R. Gastrointestinal Motility and Response to Levodopa in Parkinson's Disease: A Proof-of-Concept Study. Movement disorders: official journal of the Movement Disorder Society, 20223; 7(10): 2153–2158. https://doi.org/10.1002/mds.29176.
10. Zhou, Q., Yang, B., Zhu, Y., Wang, F., Tai, Y., Liu, Z., et al. Association of bacterial overgrowth in the small intestine with cortical thickness and functional connectivity in Parkinson's disease involving mild cognitive impairment. Brain imaging and behavior. 2024;10.1007/s11682-024-00948-w. Advance online publication. https://doi.org/10.1007/s11682-024-00948-w.
11. Dănău, A., Dumitrescu, L., Lefter, A., Tulbă, D., & Popescu, B. O. Small Intestinal Bacterial Overgrowth as Potential Therapeutic Target in Parkinson's Disease. International journal of molecular sciences. 2021; 22(21): 11663. https://doi.org/10.3390/ijms222111663.
12. Justich, M. B., Rojas, O. L., & Fasano, A. The Role of Helicobacter pylori and Small Intestinal Bacterial Overgrowth in Parkinson's Disease. *Seminars in neurology*. 2023; *43*(4): 553–561. https://doi.org/10.1055/s-0043-1771468.
13. Beckers, M., Koehler, P. J., Wanten, G. J. A., & Bloem, B. R. Berlin Bowel Bothers: Might Adolf

Hitler's Gut Problems Have Been Parkinson-Related?. European neurology. 2023; 86(3): 222–227. https://doi.org/10.1159/000530166.

14. van Kessel, S. P., Bullock, A., van Dijk, G., & El Aidy, S. (). Parkinson's Disease Medication Alters Small Intestinal Motility and Microbiota Composition in Healthy Rats. mSystems, 2022; 7(1): e0119121. https://doi.org/10.1128/msystems.01191-21.
15. Dănău, A., Dumitrescu, L., Lefter, A., Tulbă, D., & Popescu, B. O. Small Intestinal Bacterial Overgrowth as Potential Therapeutic Target in Parkinson's Disease. International journal of molecular sciences. 2021; 22(21), 11663. https://doi.org/10.3390/ijms222111663.
16. Fu, P., Gao, M., & Yung, K. K. L. Association of Intestinal Disorders with Parkinson's Disease and Alzheimer's Disease: A Systematic Review and Meta-Analysis. ACS chemical neuroscience, 2020; 11(3): 395–405. https://doi.org/10.1021/acschemneuro.9b00607.
17. Hasuike, Y., Endo, T., Koroyasu, M., Matsui, M., Mori, C., Yamadera, M., et al. Bile acid abnormality induced by intestinal dysbiosis might explain lipid metabolism in Parkinson's disease. Medical hypotheses. 2020; 134: 109436. https://doi.org/10.1016/j.mehy.2019.109436.
18. Vizcarra, J. A., Wilson-Perez, H. E., Fasano, A., & Espay, A. J. Small intestinal bacterial overgrowth in Parkinson's disease: Tribulations of a trial. Parkinsonism & related disorders. 2018; 54: 110–112. https://doi.org/10.1016/j.parkreldis.2018.04.003.
19. DiBaise, J. K., Crowell, M. D., Driver-Dunckley, E., Mehta, S. H., Hoffman-Snyder, C., Lin, T., & Adler, C. H. Weight Loss in Parkinson's Disease: No Evidence for Role of Small Intestinal Bacterial Overgrowth. Journal of Parkinson's disease. 2018; 8(4): 571–581. https://doi.org/10.3233/JPD-181386.
20. Holt P. R. Intestinal malabsorption in the elderly. Digestive Diseases (Basel, Switzerland). 2007; 25(2): 144–150. https://doi.org/10.1159/000099479.
21. Niu, X. L., Liu, L., Song, Z. X., Li, Q., Wang, Z. H., Zhang, J. L., & Li, H. H. (). Prevalence of small intestinal bacterial overgrowth in Chinese patients with Parkinson's disease. Journal of neural transmission. 2016; 123(12): 1381–1386. https://doi.org/10.1007/s00702-016-1612-8.
22. Barboza, J. L., Okun, M. S., & Moshiree, B. The treatment of gastroparesis, constipation and small intestinal bacterial overgrowth syndrome in patients with Parkinson's disease. Expert opinion on pharmacotherapy. 2015; 16(16): 2449–2464. https://doi.org/10.1517/14656566.2015.1086747.
23. Barboza, J. L., Okun, M. S., & Moshiree, B. The treatment of gastroparesis, constipation and small intestinal bacterial overgrowth syndrome in patients with Parkinson's disease. Expert opinion on pharmacotherapy, 2015; 16(16): 2449–2464. https://doi.org/10.1517/14656566.2015.1086747.
24. Fasano, A., Bove, F., Gabrielli, M., Petracca, M., Zocco, M. A., Ragazzoni, E., Barbaro, F., et al. The role of small intestinal bacterial overgrowth in Parkinson's disease. Movement disorders : official journal of the Movement Disorder Society, 2013; 28(9): 1241–1249. https://doi.org/10.1002/mds.25522.
25. Fasano, A., Visanji, N. P., Liu, L. W., Lang, A. E., & Pfeiffer, R. F. Gastrointestinal dysfunction in Parkinson's disease. The Lancet. Neurology. 2015: 14(6); 625–639. https://doi.org/10.1016/S1474-4422(15)00007-1.
26. Tan, A. H., Mahadeva, S., Thalha, A. M., Gibson, P. R., Kiew, C. K., Yeat, C. M., et al. Small intestinal bacterial overgrowth in Parkinson's disease. Parkinsonism & related disorders. 2014; 20(5): 535–540. https://doi.org/10.1016/j.parkreldis.2014.02.019.
27. Dobbs, R. J., Charlett, A., Dobbs, S. M., Weller, C., A Ibrahim, M. A., Iguodala, O., et al. Leukocyte-subset counts in idiopathic parkinsonism provide clues to a pathogenic pathway involving small intestinal bacterial overgrowth. A surveillance study. Gut pathogens, 2012; 4(1): 12. https://doi.org/10.1186/1757-4749-4-12.
28. Gabrielli, M., Bonazzi, P., Scarpellini, E., Bendia, E., Lauritano, E. C., Fasano, A., et al. Prevalence of small intestinal bacterial overgrowth in Parkinson's disease. Movement disorders : official journal of the Movement Disorder Society, 2011; 26(5): 889–892. https://doi.org/10.1002/mds.23566.
29. Hasuike Y., Endo T., Koroyasu M., Matsui M., Mori C., Yamadera M., Fujimura H., Sakoda S. Clinical features of Parkinson's disease patients with small intestinal bacterial overgrowth. Journal of the Neurological Sciences. 2017; 381: 188–373.
30. Dumitrescu, L., Marta, D., Dănău, A., Lefter, A., Tulbă, D., Cozma, L, et al. Serum and Fecal Markers of Intestinal Inflammation and Intestinal Barrier Permeability Are Elevated in Parkinson's Disease. Frontiers in neuroscience. 2021; 15: 689723. https://doi.org/10.3389/fnins.2021.689723.
31. Ostojic S. M. Inadequate Production of H_2 by Gut Microbiota and Parkinson Disease. Trends in endocrinology and metabolism: TEM. 2018; 29(5): 286–288. https://doi.org/10.1016/j.tem.2018.02.006.
32. Su, A., Gandhy, R., Barlow, C., & Triadafilopoulos, G. (). Utility of the wireless motility capsule and lactulose breath testing in the evaluation of patients with Parkinson's disease who present with functional gastrointestinal symptoms. BMJ open gastroenterology, 2017; 4(1): e000132. https://doi.org/10.1136/bmjgast-2017-000132.

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ORIGINAL ARTICLE / ÖZGÜN MAKALE

Can buttermilk (ayran) with its postbiotic content be used in the protection of colon health?

Postbiyotik içeriğiyle ayran kolon sağlığının korunmasında kullanılabilir mi?

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Abstract

Objective: In recent years, we have come across articles on the positive effects of nutrition in disease prevention and treatment processes. The microbiota formed by bacteria in the human body can play a role in various diseases and cancer. There is some information on the prevention and treatment of colon cancer by products called postbiotics produced by some bacteria in this flora. It was aimed to investigate the therapeutic effects of ayran, an ingredient rich in postbiotic products, on colon cancer.
Materials and Methods: This study evaluates the effects of postbiotic LTW 35 on normal colon fibroblast (CRL-1459) and colon cancer (CCL-224) cell lines. CRL-1459 cells treated with TT X100 for cytotoxicity and CCL-224 cells grown to sufficient density were exposed to normal buttermilk and buttermilk containing 1%, 2%, 3%, and 4% postbiotic LTW 35. Cell viability was assessed using the MTT assay, and tumor activity was measured via the Ca 19-9 tumor marker.
Results: The viability of CRL-1459 colon fibroblast cells decreases progressively with increasing concentrations of TT X100, reaching its lowest level at 0.5%. The viability of colorectal cancer cells is reduced as the concentration of postbiotic LTW 35 (*Streptococcus thermophilus* ATA-LTC St140700, *Bifidobacterium animalis* ATA-BSLA0310, *Lactobacillus acidophilus* ATA-LAP1201 ferment extract lysate) increases, with the lowest viability observed at 4%. Ca19-9 tumor marker levels in cancer cells decrease gradually with increasing concentrations of postbiotic LTW 35, showing the most significant reduction at 4%.
Conclusion: Postbiotic LTW 35-enriched buttermilk restores the viability of TTX 100-damaged normal colon fibroblast cells and reduces the viability of colorectal cancer cells in a concentration-dependent manner, indicating both restorative and anticancer effects. The observed decrease in Ca19-9 tumor marker levels further highlights its potential in reducing tumor activity.

Keywords: Cell Viability, Buttermilk (Ayran), Colon Cancer, Postbiotics

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Öz

Amaç: Son yıllarda hastalıklardan korunma ve tedavi süreçlerinde beslenmenin olumlu etkilerine ilişkin makalelere rastlıyoruz. İnsan vücudundaki bakterilerin oluşturduğu mikrobiyota çeşitli hastalıklarda ve kanserde rol oynayabiliyor. Bu florada yer alan bazı bakterilerin ürettiği postbiyotik adı verilen ürünlerle kolon kanserinin önlenmesi ve tedavisi konusunda bazı bilgiler mevcuttur. Bu çalışmada, postbiyotik ürünler açısından zengin bir bileşen olan ayranın kolon kanseri üzerindeki tedavi edici etkilerinin araştırılması amaçlanmıştır.
Gereç ve Yöntem: Bu çalışmada, postbiyotik LTW 35'in normal kolon fibroblast (CRL-1459) ve kolon kanseri (CCL-224) hücre hatları üzerindeki etkileri değerlendirilmiştir. Sitotoksisite için TT X100 ile muamele edilen CRL-1459 hücreleri ve yeterli yoğunlukta büyütülen CCL-224 hücreleri normal ayrana ve %1, %2, %3 ve %4 postbiyotik LTW 35 içeren ayrana maruz bırakılmıştır. Hücre canlılığı MTT testi kullanılarak değerlendirilmiş ve tümör aktivitesi Ca 19-9 tümör belirteci ile ölçülmüştür.
Bulgular: CRL-1459 kolon fibroblast hücrelerinin canlılığı, artan TT X100 konsantrasyonları ile düzenli olarak azalmış ve %0,5'te en düşük seviyeye ulaşmaktadır. Kolorektal kanser hücrelerinin canlılığı, postbiyotik LTW 35 (*Streptococcus thermophilus* ATA-LTC St140700, *Bifidobacterium animalis* ATA-BSLA0310, *Lactobacillus acidophilus* ATA-LAP1201 ferment ekstrakt lizatı) konsantrasyonu arttıkça azalmakta ve en düşük canlılık %4'te gözlenmektedir. Kanser hücrelerindeki Ca19-9 tümör işaretleyici seviyeleri, artan postbiyotik LTW 35 konsantrasyonları ile kademeli olarak azalmakta ve en önemli azalma %4'te görülmektedir.
Sonuç: Postbiyotik LTW 35 ile zenginleştirilmiş ayran, TTX 100 ile hasar görmüş normal kolon fibroblast hücrelerinin canlılığını geri kazandırmakta ve kolorektal kanser hücrelerinin canlılığını konsantrasyona bağlı bir şekilde azaltarak hem onarıcı hem de antikanser etkilerini göstermektedir. Ca19-9 tümör marker seviyelerinde gözlenen düşüş, tümör aktivitesini azaltma potansiyelini daha da vurgulamaktadır.

Anahtar Kelimeler: Hücre Canlılığı, Ayran, Kolon Kanseri, Postbiyotikler

INTRODUCTION

Colon cancer is a highly prevalent form of human malignant tumor, with approximately 1,148,000 new cases and 576,000 new deaths reported globally in 2020 (1). Today, surgery remains the primary treatment for patients with early-stage colon cancer. However, most colon cancer patients are diagnosed at advanced stages (2). Despite significant advances in combined treatment strategies for colon cancer, patients often face low 5-year survival rates (3). Advances in screening, surgical techniques, and adjuvant therapies led to substantial improvement in outcomes of patients diagnosed with colon cancer (4,5).

The human body harbors an estimated three trillion bacterial members that orchestrate a comprehensive interplay of physiological processes and disease susceptibilities (6). The surface of the human colonic epithelium is exposed to and interacts with highly complex and metabolically sophisticated bacterial ecosystems. The gut microbiota has an important role in maintaining a healthy human colon by protecting the host against pathogen overgrowth, shaping the development of innate and adaptive immunity, and obtaining energy and producing nutrients for the host (7). The human microbiome forms a complex multikingdom community that interacts symbiotically with its host, the human, at various sites in the body. Host-microbiome interactions influence multiple physiological processes and various multifactorial disease states (8).

The presence of abnormal changes in the intestinal microflora of colon cancer patients suggests that disruption of the intestinal microflora is closely associated with the initiation and progression of colon cancer cells (9, 10). Furthermore, probiotics have been shown to have tumor suppressive effects in colon cancer cell lines and mouse/rat tumor models (11, 12). The microbiota has been shown to produce small molecules and metabolites that have both local and systemic effects on cancer initiation, progression and treatment response (13).

Probiotics, microbiota and colon health

The gut microbiota has broad influences that contribute to the host immune system during tumor formation (14,15). The relationships between the gastrointestinal microbiota and systemic lymphoid tissues have increased interest in microbial modulation as a powerful immunotherapeutic modality. for example, modulation of the gut microbiota influences the composition of the intratumoral microbiome in pancreatic cancer, possibly through pancreatic duct communication (16-18) and modulation of gut microbiota aimed at restoring gut microbial homeostasis is becoming a potential strategy for the prevention and treatment of colon cancer (19).

Probiotics are associated with a variety of health benefits, including improving gut microflora, suppressing extreme allergic responses and tumor suppressive effects (20-22) and probiotics are defined as live microorganisms that, when administered in adequate amounts, provide health benefits to the host (23). Probiotics are now recognized to function beyond mediating the microbiota, but also to cause physiological and metabolic changes in the host (19).

Intestinal health is impaired for various reasons. Small intestinal bacterial overgrowth (SIBO), is a sickness and characterized by excessive bacterial colonization in the small intestine. There is a study on rats using probiotics by Aslan et al. and in this study he observed the important role of probiotics in the amelioration of small intestinal bacterial overgrowth (SIBO) (24). Younesi and Ayseli, in a study, stated that there is an urgent need for innovative models to strengthen functional food production processes (25). Ayseli et al. stated that the results obtained from their study constituted an important first step towards fermented foods and their health effects on various infections (26). Another important food group containing a mixture of probiotic bacteria is milk and dairy products. In a study by Bursalioglu examining the effects of human milk, mare's milk and cow colostrum milk on A549 lung cancer cell lines; the possibility that human milk may have a therapeutic role in lung cancer treatment. (27).

Postbiotics, microbiota and colon health

Postbiotic term is clearly articulated as any factor resulting from the metabolic activity of a probiotic or any released molecule that can provide beneficial effects to the host in a direct or indirect manner (28). In other words; postbiotics refer to soluble by-products and metabolites secreted by the gut microbiota that exert biological activities on the host. This term is increasingly appearing in the scientific literature and commercial products (29). In 2021, the International Scientific Probiotic and Prebiotic Society defined postbiotics as "the preparation of non-living microorganisms and/or their components that provide health benefits to the host" (29). Postbiotic preparations can be easily and stably stored at room temperature for years without the need to take into account the progressive decrease in biological activity due to the loss of bacterial viability over time (29). These functional and physical properties of postbiotics have generated great interest among researchers

(30).

Probiotic-derived ferrichrome exerts a tumour-suppressive effect via the JNK signalling pathway (31). Ferrichrome was shown to be the molecule responsible for inhibiting the progression of colon cancer cells through JNK-DDTI3-mediated apoptosis and ferricrome may be a practical anti-tumor agent that can be used to inhibit the progression of colon cancer (31). Bioactive microbial compounds such as exopolysaccharide (EPS) preparations and cell-free supernatants (CFS) from Lactobacillus species have been suggested to be bioactive in some cancers. EPS reduced the proliferation of liver and GI tumor cell lines (32), while CFS preparations derived from Lactobacillus, Bifidobacterium and Faecalibacterium species induced cellular apoptosis, reduced tumor cell proliferation and activated anti-inflammatory signaling pathways in vitro models (33). An alternative postbiotic approach could use OMVs in a tumor modulating process. A modified OMV from *E. coli* has shown promising results as a cancer immunotherapeutic agent in colorectal cancer mouse models by accumulating in tumors and producing IFNg to enhance the antitumor response within the TME (34). An OMV-containing vaccine elicited a specific antitumor immune response with elimination of lung melanoma metastasis and inhibition of subcutaneous CRC growth (35). SCFA produced from probiotic fermentation is the best known example of postbiotics (36).

Examples of some of the positive effects of the products formed by the beneficial bacteria in the microbiota content on the prevention and treatment of colon cancer. In one study, the vitamin niacin (B3 vitamin) was shown to reduce DNA damage and carcinogenesis in various cancers, including breast, colon and oral cancers (37) and niacin deficiency was significantly more prevalent in Carcinoid tumor patients (38). Short-chain fatty acids (SCFA); acetate, propionate and butyrate) are the products of anaerobic bacterial fermentation of dietary fiber in the human colon. Among SCFAs, butyrate has multiple important roles as a key in colonic epithelial homeostasis: it is the main source of energy for colonocytes (39,40); may be protective against colon carcinogenesis (41); inhibits colon carcinogenesis (42,43); promotes the growth and proliferation of normal colonic epithelial cells (44,45); stimulates fluid and electrolyte absorption (46,47). According to one theory: SCFA and butyrate affect the cell cycle by inhibiting proliferation, inducing differentiation and cell death in human cancer cells (48-50). Based on this theory, a fermented nutrition approach has been proposed as an adjuvant in colon cancer treatment. There is evidence that propionate and butyrate exert an antiproliferative effect against colon cancer cells. Butyrate and propionate are also among the most potent living metabolites that induce cell differentiation and apoptosis. They are therefore protective against colorectal cancer (49,51,52). A study by Lương and Nguyễn showed a significant association between cancer and low levels of thiamine (B1 vitamin) in serum (52). A study examining the association between folate intake levels and the incidence of colorectal cancer suggests that higher folate intake levels lead to a reduction in one of the perceived risks associated with the development of colorectal cancer (53).

Fermented dairy products are gaining more attention due to their nutritional content and the lactic acid bacteria they contain, which improve the intestinal flora (54-56). Ayran, a special type of acidic milk drink, is popular in Turkey and many countries in Asia and the Middle East (57). In various countries of the world, buttermilk (ayran) -like products are produced by adding sugar

or fruit flavors and are called drinkable yoghurt, lactic drink or fermented milk drink (58). Using healthy human colon fibroblast (ATCC CRL-1459 Colon cell line) and human colorectal cancer (ATCC -CCL-224 colorectal adenocarcinoma cell line) cell lines; treated with normal buttermilk, buttermilk containing 1%, 2%, 3% and 4% postbiotic LTW 35 (*Streptococcus thermophilus ATA-LTC St140700, Bifidobacterium animalis ATA-BSLA0310, Lactobacillus acidophilus ATA-LAP1201* ferment extract lysate) respectively. After treatment, the effects of buttermilk components on cell lines will be tested by cell viability MTT assay.

MATERIAL AND METHODS

Healthy human colon fibroblast (ATCC CRL-1459 Colon cell line) and human colorectal cancer (ATCC -CCL-224 colorectal adenocarcinoma cell line) cell lines were used. CRL-1459 colon cancer cells were cultured in DMEM medium supplemented with 8% fetal bovine serum (FBS), penicillin - potassium (48 µg/ml), streptomycin sulfate (8,000 µg mL-1), amphotericin B (25 µg mL-1) and 1.2% L-glutamine. CRL-1459 colon fibroblast cells were cultured in DMEM medium supplemented with 8% FBS, penicillin-potassium (48 µg/ml), streptomycin (8,000 µg mL-1), amphotericin B (25 µg mL-1) and 1.2% L-glutamine. Cells were grown and stocked in an incubator at 37°C with 5% CO_2 and 90% humidity until cell density reached 80%. When cell density reached the desired level, CRL-1459 normal colon fibroblast cells were treated with various levels (0.005%-0.5%) of Tritonix 100 cytotoxicity. The cell group with a viability level of 56.1% at the end of the application was used in the study. Destroyed cells were treated with normal buttermilk and buttermilk containing 1, 2, 3 and 4% postbiotic LTW 35 (*Streptococcus thermophilus ATA-LTC St140700, Bifidobacterium animalis ATA-BSLA0310, Lactobacillus acidophilus ATA-LAP1201* ferment extract lysate) respectively. ATCC -CCL-224 colon cancer cells that reached sufficient majority were treated with normal buttermilk, buttermilk containing 1%, 2%, 3% and 4% postbiotic LTW 35 (*Streptococcus thermophilus ATA-LTC St140700, Bifidobacterium animalis ATA-BSLA0310, Lactobacillus acidophilus ATA-LAP1201* ferment extract lysate), respectively. After the treatment, cell viability was tested with MTT and CA 19-9 tumor marker was detected from cell lysates as a confirmation test.

RESULTS

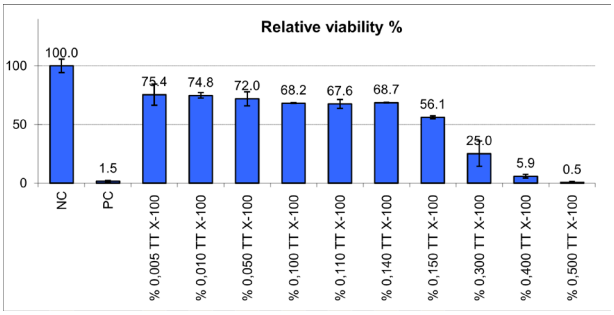

Figure 1. Viability graph of cells destroyed by cytotoxicity

Figure 1. shows the decrease in viability of CRL-1459 colon fibroblast cells after exposure to different concentrations (0.005%-0.5%) of TT X100. The graph indicates that as the concentration of TT X100 increases, the cell viability decreases significantly.

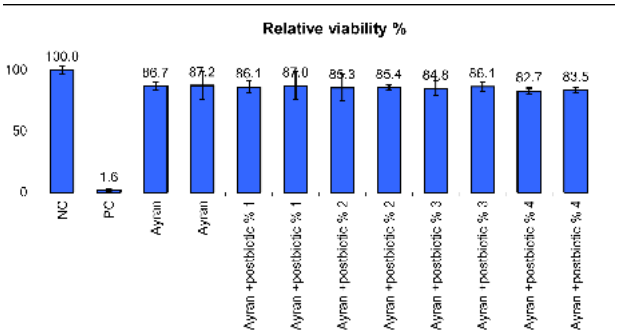

Figure 2. Viability graph after buttermilk treatment of cells destroyed by cytotoxicity

Figure 2 and figure (5-16) shows the viability rates of CRL-1459 colon fibroblast cells damaged by cytotoxicity after being treated with normal buttermilk and buttermilk containing postbiotic LTW 35. It is observed that the application of buttermilk and postbiotic content increased cell viability, and this effect is related to the concentrations used.

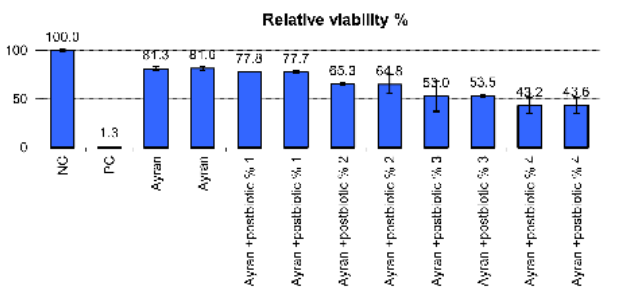

Figure 3. Viability graph after treatment of tumor cells

Figure 3 and figure (5-16) shows the viability rates of ATCC CCL-224 colorectal cancer cells after being treated with normal buttermilk and buttermilk containing 1%, 2%, 3%, and 4% postbiotic LTW 35. The applied buttermilk and postbiotic content reduced the viability of cancer cells, demonstrating potential anticancer effects.

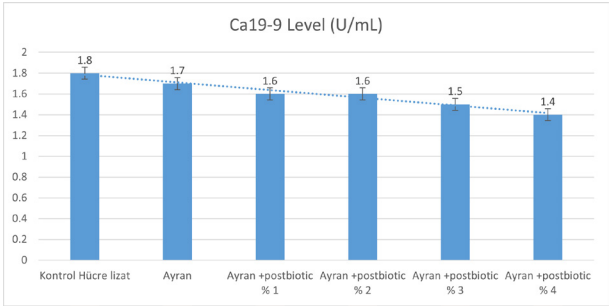

Figure 4. Ca 19-9 results graph

Figure 4. shows the changes in Ca 19-9 tumor marker levels in cell lysates obtained from ATCC CCL-224 colorectal cancer cells after treatment with normal buttermilk and buttermilk containing 1%, 2%, 3%, and 4% postbiotic LTW 35. The graph demonstrates a gradual decrease in Ca 19-9 levels as the postbiotic concentration increases, indicating the potential antitumor effect of the postbiotic.

Figure 5. Microscope view of destroyed cells

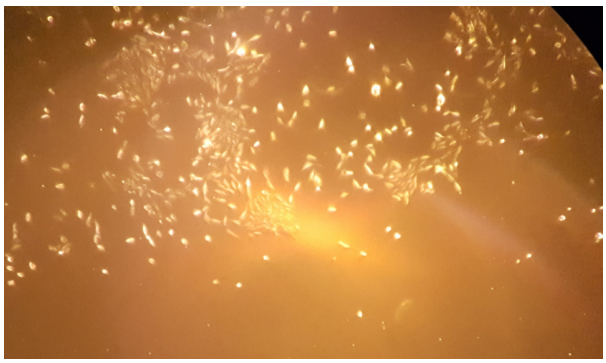

Figure 6. Microscope image of buttermilk-treated cells

Figure 7. Microscope image of buttermilk + 1% postbiotic-treated cells

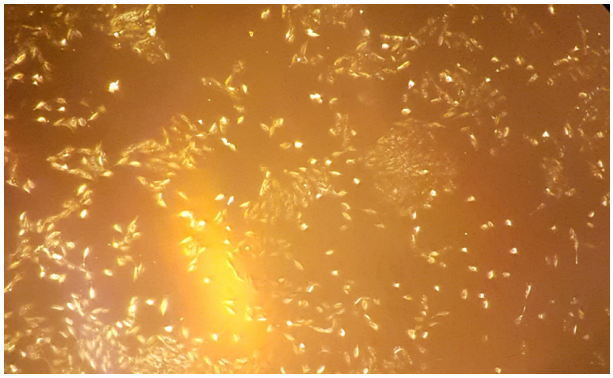

Figure 8. Microscope image of buttermilk + 2% postbiotic-treated cells

Figure 9. Microscope image of buttermilk + 3 % postbiotic-treated cells

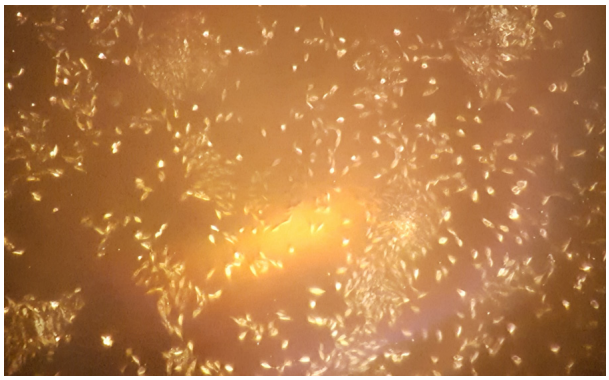

Figure 10. Microscope image of buttermilk + 4% postbiotic-treated cells

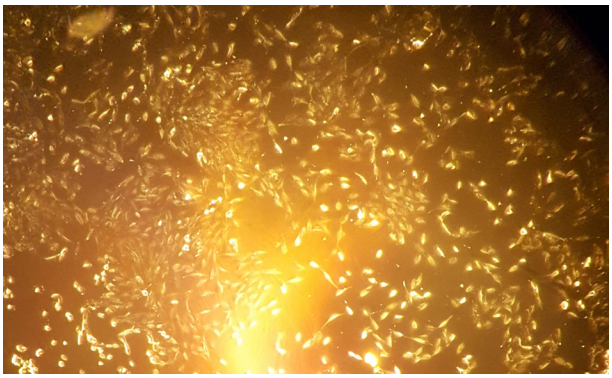

Figure 11. Microscope image of colon cancer cells

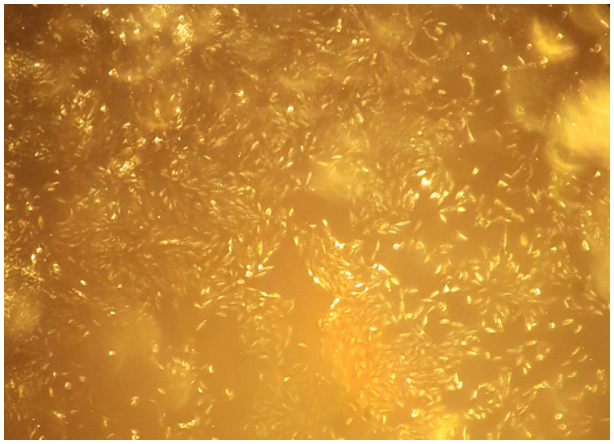

Figure 12. Microscope image of colon cancer cells after buttermilk supplementation

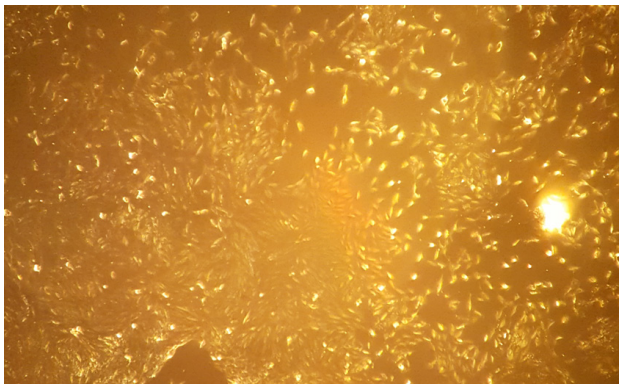

Figure 13. Microscope image of colon cancer cells after buttermilk and 1% postbiotic supplementation

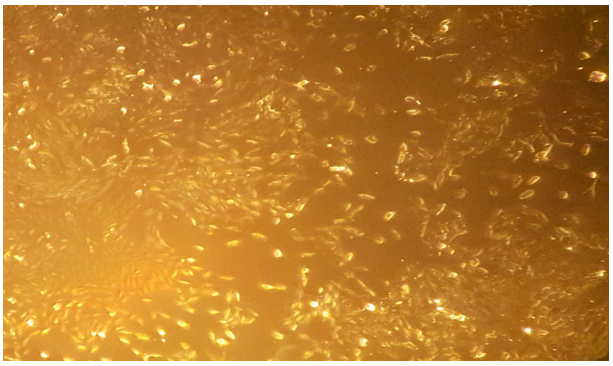

Figure 14. Microscope image of colon cancer cells after buttermilk and 2% postbiotic supplementation

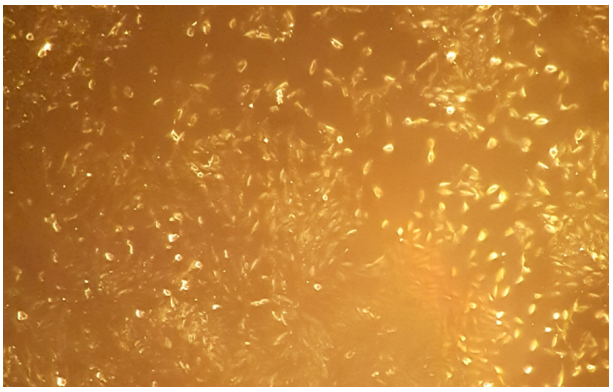

Figure 15. Microscope image of colon cancer cells after buttermilk and 3% postbiotic supplementation

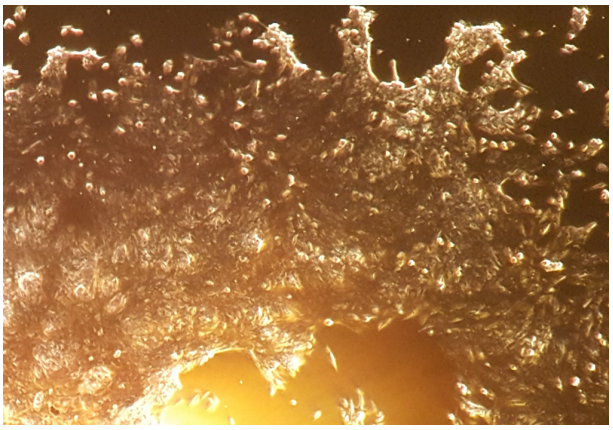

Figure 16. Microscope image of colon cancer cells after buttermilk and 4% postbiotic supplementation

DISCUSSION

The purpose of this study is to examine the effects of normal buttermilk and postbiotic LTW 35 (*Streptococcus thermophilus ATA-LTC St140700, Bifidobacterium animalis ATA-BSLA0310, Lactobacillus acidophilus ATA-LAP1201* ferment extract lysate) -enriched buttermilk on the viability of colon cancer cells. It also investigates changes in Ca 19-9 tumor marker levels to explore their potential cytoprotective and anticancer activities. Figure 1 highlights the dose-dependent decrease in the viability of CRL-1459 colon fibroblast cells after exposure to TTX 100. Figure 2 and figure (5-16) shows the improvement in viability of cytotoxicity-damaged fibroblast cells following treatment with buttermilk and postbiotic LTW 35, with effects linked to concentration levels. Figure 3 and figure (5-16) demonstrates that buttermilk and postbiotic LTW 35 reduce the viability of colorectal cancer cells, suggesting anticancer properties. Figure 4 and figure (5-16) illustrates a gradual decrease in Ca 19-9 tumor marker levels in cancer cells treated with buttermilk containing postbiotics, indicating the potential antitumor effects of the postbiotic. A study conducted by Bursalioglu (2021), which examined milk groups rich in probiotics, the effects of lyophilized human milk, mare milk, and cow colostrum on A549 lung cancer and MRC5 healthy lung cell lines were evaluated. The study found that human milk exhibited the strongest anticancer effects, followed by mare milk, while cow colostrum showed the least effect. Human and mare milk demonstrated antiproliferative effects on cancer cells without causing harm to healthy cells (27). Milk products contain a large number of probiotic bacteria and metabolites. The release of this content during the fermentation of probiotic bacteria in dairy products may prevent colorectal carcinogenesis (59). Moreover, van't Veer et al. hypothesized that high consumption

of fermented milk products (predominantly yogurt and buttermilk) may create protection against breast cancer (60). This study confirmed the effects of products containing the following ATA-coded postbiotics according to the literature. A study conducted by Aslan and colleagues demonstrated that postbiotics specifically inhibit odor-causing microorganisms while supporting and balancing the natural axillary microbiota. The findings revealed that formulations containing Lactobacillus ferment lysate extract were effective in reducing unpleasant odors by normalizing the microbiota (61). A study conducted by Gokce and Aslan investigated the antimicrobial potential of liposomal postbiotics in gel formulations. The optimized gel (LG1) showed effective antimicrobial activity against various pathogens, comparable to free postbiotics, while providing advantages such as controlled release, stability, and enhanced usability. These findings highlight the potential of liposomal postbiotics for pharmaceutical applications (62).

CONCLUSION

Increasing concentrations of TTX 100 significantly reduce the viability of CRL-1459 colon fibroblast cells, confirming a dose-dependent cytotoxic effect. Treatment with postbiotic LTW 35-enriched buttermilk improves the viability of cytotoxicity-damaged colon fibroblast cells, with higher concentrations providing greater restorative effects. Postbiotic LTW 35 reduces the viability of colorectal cancer cells in a concentration-dependent manner, demonstrating its potential anticancer properties. The observed decrease in Ca 19-9 tumor marker levels with postbiotic LTW 35 treatment highlights its potential role in reducing tumor activity in colorectal cancer cells.

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Conflict of interest statement: The authors report no conflict of interest.

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Ethical Decleration: No need.

Author Contribution: Concept: EB, LTÇ,BÇ, NG, HTK, İA, Design: EB, LTÇ,BÇ, NG, HTK, İA, Data Collection or Processing: G.K., Z.E., H.İ.K., H.E., Analysis or Interpretation: EB, LTÇ,BÇ, NG, HTK, İA, Literature Search: EB, LTÇ,BÇ, NG, HTK, İA, Writing: EB, LTÇ,BÇ, NG, HTK, İA

REFERENCES

1. Sung H, et al. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries: CA Cancer J. Clin. 2021; 71(3): 209–249
2. Harris TJ and McCormick F. The molecular pathology of cancer: Nat. Rev. Clin. Oncol. 2010; 7(5): 251–265
3. Haggar FA and Boushey RP, Colorectal cancer epidemiology: Incidence, mortality, survival, and risk factors: Clin. Colon Rectal Surg. 2009; 22(4): 191–197
4. Cho JR, et al. Effectiveness of oral fluoropyrimidine monotherapy as adjuvant chemotherapy for high-risk stage II colon cancer: Ann. Surg. Treat. Res. 2022; 102: 271–280
5. Miller KD, et al. Cancer treatment and survivorship statistics, 2022: CA Cancer J. Clin. 2022; 72: 409–436
6. Sender R, Fuchs S, and Milo R. Revised estimates for the number of human and bacteria cells in the body: PLoS Biol. 2016; 14: e1002533
7. Hooper LV, Littman DR and Macpherson AJ, Interactions between the microbiota and the immune system: Science. 2012; 336:1268–1273
8. Cullin N, Antunes CA, Straussman R, Stein-Thoeringer CK, and Elinav E, Microbiome and cancer: Cancer Cell. 2021; 39: 1320
9. Marchesi JR, et al. Towards the human colorectal cancer microbiome: PLoS ONE 2011; 6: e20447
10. Sobhani I, et al. Microbial dysbiosis in colorectal cancer (CRC) patients: PLoS ONE. 2011; 6: e16393
11. Jan G, et al. Propionibacteria induce apoptosis of colorectal carcinoma cells via short-chain fatty acids acting on mitochondria: Cell Death Differ. 2002; 9:179–188
12. Cousin FJ, et al. Milk fermented by Propionibacterium freudenreichii induces apoptosis of HGT-1 human gastric cancer cells: PLoS ONE. 2012; 7:e31892
13. Elinav E, Garrett WS, Trinchieri G, and Wargo J. The cancer microbiome. Nature Reviews Cancer. 2019;

(19):371-376

14. Zitvogel L, Ma Y, Raoult D, Kroemer G, Gajewski TF, The microbiome in cancer immunotherapy: Diagnostic tools and therapeutic strategies: Science. 2018; 359:1366–1370
15. Honda K, Littman DR, The microbiota in adaptive immune homeostasis and disease: Nature. 2016; 535:75–84
16. Riquelme E, et al. Tumor microbiome diversity and composition influence pancreatic cancer outcomes: Cell. 2019; 178:795–806.e12
17. Aykut B, et al, The fungal mycobiome promotes pancreatic oncogenesis via activation of MBL: Nature. 2019; 574:264–267
18. Pushalkar S, et al. The pancreatic cancer microbiome promotes oncogenesis by induction of innate and adaptive immune suppression: Cancer Discov. 2018; 8:403–416
19. Fong W, Li Q, Yu J, Gut microbiota modulation: a novel strategy for prevention and treatment of colorectal cancer: Oncogene. 2020; 39:4925–4943
20. Isolauri E, et al. Nutrition, Allergy, Mucosal Immunology, and Intestinal Microbiota (NAMI) Research Group Report. Probiotics: use in allergic disorders: a Nutrition, Allergy, Mucosal Immunology, and Intestinal Microbiota (NAMI) Research Group Report: J. Clin. Gastroenterol. 2008; 42:S91–S96
21. Kallioma¨ki M, et al. Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial: Lancet. 2001; 357:1076–1079
22. Rowland IR, et al. Effect of Bifidobacterium longum and inulin on gut bacterial metabolism and carcinogen-induced aberrant crypt foci in rats: Carcinogenesis. 1998; 19: 281–285
23. Geier MS, Butler RN, Howarth GS. Probiotics, prebiotics and synbiotics: a role in chemoprevention for colorectal cancer? Cancer Biol Ther. 2006;5:1265–9
24. Aslan I, Tarhan Celebi L, Kayhan H, Kizilay E, Gulbahar MY, Kurt H and Cakici B. Probiotic Formulations Containing Fixed and Essential Oils Ameliorates SIBO-Induced Gut Dysbiosis in Rats: Pharmaceuticals. 2023; 16:1041
25. Erfan Younesi E, Ayseli MT.2015. An integrated systems-based model for substantiation of health claims in functional food development. Trends in Food Science & Technology 41(1),95-100.
26. Ayseli MT, Coskun I, Selli S. 2023, Evaluation of volatile and thermal properties of boza, a traditional fermented beverage. Microchemical Journal, 193, 108918.
27. Bursalioglu EO. Effect of Cow Colostrum, Mare Milk, and Human Milk on the Viability of Lung Healthy and Cancer Cell Lines. The Iranian Red Crescent Medical Journal (IRCMJ) 2021; 23 (5)
28. Tsilingiri K, and Rescigno M. Postbiotics: what else? Benef. Microbes. 2013; 4:101–107
29. Salminen S, et al. The International Scientific Association of Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics: Nat. Rev. Gastroenterol. Hepatol. 2021; 18:649–667
30. Sabahi S, et al. Postbiotics as the new frontier in food and pharmaceutical research: Crit. Rev. Food Sci. Nutr. 2022; 1–28
31. Konishi H, Fujiya M, Tanaka H, Ueno N, Kentaro Moriichi K, Sasajima J, Ikuta K, Akutsu H, Tanabe H & Kohgo Y. Probiotic-derived ferrichrome inhibits colon cancer progression via JNK-mediated apoptosis: natüre communications. 2016; 7:12365
32. Wang K, Li W, Rui X, Chen X, Jiang M, and Dong M. Characterization of a novel exopolysaccharide with antitumor activity from Lactobacillus plantarum 70810: Int. J. Biol. Macromol. 2014; 63:133–139
33. Homayouni RA, Maleki AL, Samadi KH, Zavoshti HF, and Abbasi A. Postbiotics as promising tools for cancer adjuvant therapy: Adv. Pharm. Bull. 2021; 11:1–5
34. Kim OY, Park HT, Dinh NTH, Choi SJ, Lee J, Kim JH, Lee SW, and Gho YS. Bacterial outer membrane vesicles suppress tumor by interferon- gamma-mediated antitumor response: Nat. Commun. 2017; 8:626
35. Cheng K, Zhao R, Li Y, Qi Y, Wang Y, Zhang Y, Qin H, Qin Y, Chen L, Li C, et al. Bioengineered bacteria-derived outer membrane vesicles as a versatile antigen display platform for tumor vaccination via Plugand-Display technology: Nat. Commun. 2021; 12:2041
36. Konstantinov SR, Kuipers EJ, Peppelenbosch MP. Functional genomic analyses of the gut microbiota for CRC screening: Nat Rev Gastroenterol Hepatol. 2013;10:741
37. Surjana D, Halliday GM, Damian DL. Role of nicotinamide in DNA damage, mutagenesis, and DNA repair: J Nucleic Acids. 2010; 157591
38. Shah GM, Shah RG, Veillette H, Kirkland JB, PasiekaJL, Warner RRP. Biochemical Assessment of Niacin Deficiency Among Carcinoid Cancer Patients: American Journal of Gastroenterology. 2005;100 (10): 2307-2314
39. Roediger WE. Role of anaerobic bacteria in the metabolic welfare of the colonic mucosa in man: Gut. 1980; 21:793–798
40. Scheppach W, Bartram P, Richter A, Richter F, Liepold H, et al. Effect of short-chain fatty acids on the human colonic mucosa in vitro: J Parenter Enteral Nutr. 1992; 16:43–48
41. Scheppach W, Bartram HP, and Richter F. 1995. Role of short-chain fatty acids in the prevention of colorectal cancer: Eur. J. Cancer. 1080; 31A: 1077

42. Hague A, Elder DJ, Hicks DJ, and Paraskeva C. Apoptosis in colorectal tumour cells: induction by the short-chain fatty acids butyrate, propionate and acetate and by the bile salt deoxycholate: Int J Cancer. 1995; 60:400–406
43. Heerdt BG, Houston MA, and Augenlicht LH. Potentiation by specific short-chain fatty acids of differentiation and apoptosis in human colonic carcinoma cell lines: Cancer Res. 1994; 54:3288–3293
44. Kripke SA, Fox AD, Berman JM, Settle RG, and Rombeau JL. Stimulation of intestinal mucosal growth with intracolonic infusion of short-chain fatty acids: J Parent Enter Nutr. 1989; 13:109–116, 10
45. Sakata T and von Engelhardt W. Stimulatory effect of short-chain fatty acids on the epithelial cell proliferation in rat large intestine: Comp Biochem Physiol. 1983; 74A:459–462
46. Montrose MH and Kere J. Anion absorption in the intestine: anion transporters, short chain fatty acids, and role of the DRA gene product: Curr Top Membr Transp. 2001; 50:301–328
47. Binder HJ and Mehta P. Short-chain fatty acids stimulate Na and Cl absorption in vitro in the rat distal colon: Gastroenterology. 1989; 96: 989–996
48. Siavoshian S, Segain JP, Kornprobst M, Bonnet C, Cherbut C, Galmiche JP, Blottiere HM. Butyrate and trichostatin A effects on the proliferation/ differentiation of human intestinal epithelial cells: induction of cyclin D3 and p21 expression: Gut. 2000; 46(4): 507–514
49. Aoyama M, Kotani J, Usami M. Butyrate and propionate induced activated or non-activated neutrophil apoptosis via HDAC inhibitor activity but without activating GPR-41/GPR-43 pathway: Nutrition. 2010; 26(6): 653–661
50. Tang Y, Chen Y, Jiang H, Nie D. Short-chain fatty acids induced autophagy serves as an adaptive strategy for retarding mitochondriamediated apoptotic cell death: Cell Death Differ. 2011a;18(4): 602–618
51. Pool-Zobel BL, Sauer J. Overview of experimental data on reduction of colorectal cancer risk by inulin-type fructans: J Nutr 2007;137(11 Suppl):2580S–2584S
52. Lương KVQ and Nguyễn LTH. The Role of Thiamine in Cancer: Possible Genetic and Cellular Signaling Mechanisms: Cancer Genomıcs & Proteomıcs. 2013; 10: 169-186
53. Kennedy DA, Stern SJ, Moretti M, Sarkar IM, Nickel C, Koren G. Folate intake and the risk of colorectal cancer: A systematic review and meta-analysis: Cancer Epidemiology. 2011; (35)1: 2-10
54. Shangpliang HNJ, Rai R, Keisam S, Jeyaram K, Tamang JP. Bacterial community in naturally fermented milk products of Arunachal Pradesh and Sikkim of India analysed by high-throughput amplicon sequencing: Sci. Rep. 2018; 8:1532
55. Turkmen N, Akal C, Ozer B. Probiotic dairy-based beverages: a review: J. Funct. Foods. 2019; 53: 62–75
56. Wu H, Hulbert GJ, Mount JR. Effects of ultrasound on milk homogenization and fermentation with yoghurt starte: Innovative Food Sci. Emerg. Technol. 2001; 1: 211–218
57. Koksoy A, Kilic M. Use of hydrocolloids in textural stabilization of a yoghurt drink, ayran: Food Hydrocolloids. 2004;18: 593–600
58. Yalçın S. Homojenizasyon ve ısıl işlem uygulamalarının farklı oranlarda yağ içeren sütlerden üretilen ayranın fizikokimyasal ve duyusal özellikleri üzerine etkisinin belirlenmesi, Akdeniz Üniversitesi: Fen Bilimleri Enstitüsü Yüksek Lisans Tezi, 2016.
59. Ebringer L, Ferencík M, Krajcovic J. Beneficial health effects of milk and fermented dairy products--review. Folia Microbiol (Praha). 2008;53(5):378-94. doi: 10.1007/s12223-008-0059-1.
60. van›t Veer P, Dekker JM, Lamers JW, Kok FJ, Schouten EG, Brants HA, et al. Consumption of fermented milk products and breast cancer: a case-control study in The Netherlands. Cancer Res. 1989;49(14):4020-3.
61. Aslan I and Tarhan Celebi L., Postbiotics Cosmetic Formulation: In Vitro Efficacy Studies on a Microbiome Friendly Antiperspirant, J Res Pharm. 2023; 27(5): 2095-2105.
62. Gokce HB, and Aslan I, Novel Liposome–Gel Formulations Containing a Next Generation Postbiotic: Characterization, Rheological, Stability, Release Kinetic, and In Vitro Antimicrobial Activity Studies, Gels 2024, 10, 746.

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ORIGINAL ARTICLE / ÖZGÜN MAKALE

Novel microbiome friendly purifying oil cleanser formulation with oil-soluble postbiotics

Yağda çözünen postbiyotiklerle yeni mikrobiyom dostu temizleyici yağ formülasyonu

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Abstract

Objective: In recent years, the field of preventive cosmetic care has gained importance alongside the established effectiveness of nutrition in preventing diseases. Within the scope of this preventive approach, the microbiota has been a focal point in numerous studies, with its state being associated with a range of health concerns, including inflammation, metabolic diseases, dermatological diseases, and neurodegenerative diseases.The present study aims to investigate the positive effects of a cleanser formulated with a postbiotic raw material dissolved in oil on skin microbiota and cells. In this study, the skin cleansing oil containing 2% postbiotic LTO 35 was utilised as a skin microbiota simulation (Lactobacillus crispatus LTC-KC24011, Staphylococcus epidermidis ATA-LSE 0198052) and normal keratinocytes (HEKA 500K CELL), Staphylococcus aureus ATA-LTCA 011204, Staphylococcus capitis ATA-LSC 0201201, Propionibacterium acnes ATA-LPC 0204221, Streptococcus pyogenes ATA-TCSP 210911, and Candida albicans ATA-LTCA 0504212. The results demonstrated that the cleanser did not cause harm to keratinocytes, maintained microbiota balance, and promoted cellular repair.The objective of this study was to investigate the positive effects of a cleanser made using oil-solubilised postbiotic raw material on skin microbiota and cells.
Material&Method: In this study, a skin cleansing oil containing 2% postbiotic LTO 35 was utilised to simulate normal keratinocytes (HEKA 500K CELL) and skin microbiota (Lactobacillus crispatus LTC-KC24011, Staphylococcus epidermidis ATA-LSE 0198052, Staphylococcus aureus ATA -LTCA 011204, Staphylococcus capitis ATA-LSC 0201201, Propionibacterium acnes ATA-LPC 0204221, Streptococcus pyogenes ATA TCSP 210911, Candida albicans ATA-LTCA 0504212. The bacteria utilised in the simulation and the postbiotic employed in the formulation (LTO 35 Streptococcus thermophilus ATA-LTC St140700, Bifidobacterium animalis ATA-BSLA0310, Lactobacillus acidophilus ATA-LAP1201 ferment extract in oil lysate) were obtained from the ATA BIO Technology culture collection. The simulation contact time was applied as 1 hour.
Results: The Purifying Oil Cleanser with Postbiotics sample examined in this study was found to be microbiologically suitable. The equilibrium test yielded a positive result, and it was observed that the sample did not change in the direction of pathogens. According to the analyses, it can be concluded that the Purifying Oil Cleanser with Postbiotics sample does not harm the diversity of skin microbiota and is microbiome-friendly.
Conclusion: Upon evaluation of the results, it was observed that the substance did not cause harm to keratinocytes, it maintained microbiota balance, and it promoted cellular repair.
Keywords: Probiotic, Postbiotic, Microbiota Friendly, Oil Cleanser, Cosmetic

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Öz

Amaç: Son yıllarda hastalıklardan korunmada beslenmenin etkinliğinin yanında koruyucu kozmetik bakım da giderek önem kazanmıştır. Koruyucu yaklaşımda özellikle mikrobiyotanın etkinliği pek çok çalışmada yer almıştır. Mikrobiyotanın durumu, vücuttaki enflamasyon, çeşitli metabolik hastalıklar, dermal hastalıklar, nörodejeneratif hastalıklar veya dermal hastalıklarla ilişkilendirilmiştir. Bu çalışmada, yağ içinde çözündürülmüş postbiyotik hammadde kullanılarak yapılan temizleyicinin cilt mikrobiyotasına ve hücre üzerine olumlu etkilerinin araştırılması amaçlanmıştır. Bu çalışmada, %2 postbiyotik LTO 35 içeren cilt temizleyici yağın, normal keratinositler (HEKA 500K CELL) ve cilt mikrobiyota simülasyonu (Lactobacillus crispatus LTC-KC24011, Staphylococcus epidermidis ATA-LSE 0198052, Staphylococcus aureus ATA-LTCA 011204, Staphylococcus capitis ATA -LSC 0201201, Propionibacterium acnes ATA-LPC 0204221, Streptococcus pyogenes ATA TCSP 210911, Candida albicans ATA-LTCA 0504212) üzerindeki etkileri değerlendirilmiştir. Sonuçlar değerlendirildiğinde, keratinositlere zarar vermediği, mikrobiyota dengesini koruduğu ve hücresel onarıma destek olduğu görülmüştür. Bu çalışmada, yağ içinde çözündürülmüş postbiyotik hammadde kullanılarak yapılan temizleyicinin cilt mikrobiyotasına ve hücre üzerine olumlu etkilerinin araştırılması amaçlanmıştır.

Yöntem: Bu çalışmada, %2 postbiyotik LTO 35 içeren cilt temizleyici yağın, normal keratinositler (HEKA 500K CELL) ve cilt mikrobiyota simülasyonu (Lactobacillus crispatus LTC-KC24011, Staphylococcus epidermidis ATA-LSE 0198052, Staphylococcus aureus ATA-LTCA 011204, Staphylococcus capitis ATA -LSC 0201201, Propionibacterium acnes ATA-LPC 0204221, Streptococcus pyogenes ATA TCSP 210911, Candida albicans ATA-LTCA 0504212) kullanılmıştır. Simülasyonda kullanılan bakteriler ve formülasyon içinde kullanılan postbiyotik (LTO 35 Streptococcus thermophilus ATA-LTC St140700, Bifidobacterium animalis ATA-BSLA0310, Lactobacillus acidophilus ATA-LAP1201 ferment ekstrakt in oil lizat) ATA BIO Teknoloji kültür koleksiyonundan temin edilmiştir. Simülasyon kontak süresi 1 saat olarak uygulanmıştır.

Bulgular: Bu çalışmada kullanılan Purifying Oil Cleanser with Postbiotics numunesi, mikrobiyolojik açıdan uygun olarak değerlendirilmiştir. Denge testi olumlu bulunup, patojen yönünde değişmediği gözlenmiştir. Yapılan analizlere göre; Purifying Oil Cleanser with Postbiotics numunesinin cilt mikrobiyotası çeşitliliğine zarar vermediği ve mikrobiyom dostu olduğu ifade edilebilir.

Sonuç: Sonuçlar değerlendirildiğinde, keratinositlere zarar vermediği, mikrobiyota dengesini koruduğu ve hücresel onarıma destek olduğu görülmüştür.

Anahtar Kelimeler: Probiyotik, Postbiyotik, Mikrobiyota Dostu, Yağ Temizleyici, Kozmetik

INTRODUCTION

In recent years, research has increasingly highlighted the role of cutaneous microbiota in skin health and disease. Historically, cosmetic products were formulated for objectives such as cleansing, hydration, or protection against external factors; however, contemporary approaches that target the maintenance or enhancement of skin microbiota are gaining significance (1). In this context, the use of microbiota-focused ingredients such as prebiotics, probiotics, and postbiotics is of interest in the cosmetics sector.

Probiotics are defined as live microorganisms that provide benefits to the host, while prebiotics are selectively fermentable ingredients that support the growth or activity of the microbiota (2). Postbiotics are metabolic products of cellular components produced or secreted by probiotic microorganisms that do not require the presence of live microorganisms and provide benefits to the host (3). Postbiotics offer a more stable, standardisable, and safer alternative to the use of probiotics (3, 4).

Paraprobiotics (also termed inactive probiotics or "ghost" probiotics) and postbiotics represent alternative approaches to traditional probiotic use. Paraprobiotics are inactivated microbial cells or cell lysates that can provide host benefits when administered at adequate concentrations, while postbiotics are bioactive. Postbiotics are bioactive molecules (e.g., metabolites

and cellular fractions) synthesized by probiotic microorganisms that confer physiological benefits to the host. These approaches aim to overcome the limitations related to the viability, stability, and safety profile of probiotics and offer the potential for microbiome-targeted interventions (5,6).

The benefits of using postbiotics can be direct or indirect. For instance, postbiotics have been shown to maintain microecological and immune homeostasis by enhancing the interaction of the human organism with the microbiota (7, 8). Furthermore, positive effects on mental health can be achieved by improving the functioning of the brain-gut-microbiota axis. Postbiotics have also been demonstrated to exert direct benefits on host cells, while indirect benefits include the promotion of probiotics and the inhibition of pathogen growth. As with prebiotics, the effects of postbiotics vary depending on the type, strain and metabolic product of the microorganism (9). The most important effects of the postbiotic product SCFA are its anti-inflammatory and antioxidant properties. The present study sought to evaluate the effects of postbiotic LTO 35, obtained from *S. thermophilus, B. animalis and L. acidophilus* lysates used in an oil-based cleanser formulation, on human keratinocyte cells and simulated skin microbiota in *in vitro* models.

A study conducted by Aslan & Tarhan Celebi (2023) demonstrated that postbiotics specifically inhibit odour-causing microorganisms while concurrently supporting and balancing the natural axillary microbiota. The findings revealed that formulations containing *Lactobacillus* ferment lysate extract were effective in reducing unpleasant odours by normalising the microbiota (10). In a study conducted by Gökçe and Aslan on the pharmaceutical applications of postbiotics, the antimicrobial potential of liposomal postbiotics in gel formulations was investigated. The optimised gel (LG1) demonstrated effective antimicrobial activity that was comparable to that of free postbiotics against various pathogens, while providing advantages such as controlled release, stability and improved usability. These findings emphasise the potential of liposomal postbiotics for pharmaceutical applications (11). In a study by Tarhan Celebi et al., the treatment of colon cancer by postbiotic substances is examined. The viability of normal colon fibroblast cells damaged by TTX 100 is restored by buttermilk enriched with the postbiotic LTW 35, and the viability of colorectal cancer cells is reduced in a concentration-dependent manner, showing both reparative and anticancer effects. The observed decrease in Ca19-9 tumour marker levels further emphasises its potential to reduce tumour activity (12).

A plethora of academic studies have been conducted in the domain of nutricosmetics and cosmetics, encompassing a wide range of natural sources (13,14), essential oils (15) from hair care to skin care (16, 17). Moreover, even in the field of baby care, there exist studies that utilize natural sources and investigate their antimicrobial properties (18, 19). In addition to natural products, safety studies such as ADME (absorption, distribution, metabolism, excretion) of many semi-synthetic and fully synthetic active substances (20, 21) and studies on antibacterial activity have been conducted (22). However, these studies do not address

the subject of microbiome-friendly research on intestine, skin and skin microbiota.

The skin, the largest organ of the human body, is colonised by a variety of microorganisms. The majority of these microorganisms are harmless and even beneficial to their hosts. This colonisation is driven by the ecology of the skin surface, which is highly variable depending on environmental factors. In addition, the microbiota also functions in the education of the immune system (23). Keratinocytes in the skin represent a cellular compartment that is constantly renewed and the wound healing process is dominated by the interaction of keratinocytes with fibroblasts (24,25,26).

The practice of cleaning is an important daily activity that is associated with both skin diseases and general health. It is a relatively modern concept, coinciding with the mass use of commercial soap from the early 20th century (27). It is estimated that the bacteria in the human body far outnumber the human cells in an individual, and this community is termed the human microbiome. According to another view, various microbial communities that have fundamental roles in human health and disease are present in the human body (28).

The present study set out to evaluate the effects of postbiotic LTO 35, obtained from *S. thermophilus, B. animalis* and *L. acidophilus* lysates used in an oil-based cleanser formulation, on human keratinocyte cells and simulated skin microbiota in vitro models.

METHODS

In this research, a skin cleansing oil containing 2% postbiotic LTO 35 was utilised to simulate normal keratinocytes (HEKA 500K CELL) and skin microbiota (*Lactobacillus crispatus* LTC-KC24011, *Staphylococcus epidermidis* ATA-LSE 0198052, *Staphylococcus aureus* ATA-LTCA 011204, *Staphylococcus capitis* ATA -LSC 0201201, *Propionibacterium acnes* ATA-LPC 0204221, *Streptococcus pyogenes* ATA TCSP 210911, *Candida albicans ATA-LTCA 0504212*). The bacteria utilised in the simulation and the postbiotic employed in the formulation (LTO 35 *Streptococcus thermophilus* ATA-LTC St140700, *Bifidobacterium animalis* ATA-BSLA0310, *Lactobacillus acidophilus* ATA-LAP1201 ferment extract in oil lyzate) were obtained from the ATA BIO Technology culture collection in Türkiye. The duration of contact in the simulation was set at one hour.

MTT (In *vitro* cytotoxicity fest)

Tetrazolium salts, such as MTT, are frequently utilised in cell proliferation tests, which are based on the measurement of metabolic activity. The underlying principle is based on the measurement of the colour change in the absorption spectrum by an ELISA reader or a spectrophotometer, as a result of the increased dehydrogenase enzyme activity of proliferating cells. In this process, tetrazolium (MTT: yellow) is used to produce formazan (purple) dye. The cells will be cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco) containing 10% fetal bovine serum (FBS, Gibco) and 1% penicillin/streptomycin in an oven with 5% CO2 maintained at 37°C. When

cells reach 80% proliferation, they will be washed with phosphate-buffered saline (PBS) and trypsinized with 0.25% Trypsin-EDTA for passaging and seeding each time. Cells reaching sufficient proliferation will be used in the subsequent toxicity tests.

Table 1. Microorganisms representing Artificial Skin Microbiota and amount of utilisation.

Microorganism	İnoculum
Lactobacillus crispatus ATA-LTC 240522	3.50E+02
Staphylococcus epidermidis ATA-LSE 0198052	3.50E+02
Staphylococcus aureus ATA-LTCA 011204	3.50E+02
Staphylococcus capitis ATA -LSC 0201201	3.50E+02
Cutibacterium (Propionibacterium) acnes ATA-LPC 0204221	3.50E+02
Streptococcus pyogenes ATA TCSP 210911	3.50E+02
Candida albicans ATA-LTCA 0504212	3.50E+02

The environment has been modified to be conducive to the presence of artificial microbiomes.

Microbiological analyses

The formulation developed for microbiological analysis within the scope of the study was disinfected with 70% ethanol. To dissolve the product, 5 g/L polysorbate 80 was added to 90 ml TSP (Buffered Sodium Chloride Peptone), 10 g of sample was added and left to dissolve in a water bath for 10-15 minutes. Serial dilutions (10^{-2}, 10^{-3}, 10^{-4}) were prepared by transferring 1 mL of the sample suspension to 9 mL of TSP using the pour plate method. The dilutions were repeated twice by inoculating 1 mL of the diluted tubes into a 90 mm petri dish. Then 15-17 mL of agar medium cooled to 45°C in a water bath was poured into the petri dishes and left to freeze. Tryptic Soy Agar (TSA) was used for the total number of aerobic mesophilic microorganisms which were left at 30-35°C for 3-5 days. SDA medium was used for total yeast and mould counts and the media were incubated at 20-25°C for 5-7 days. In case of growth, the calculation formula is used to count colonies visible to the naked eye. This formula is as follows:

CFU/ml = Total number of colonies obtained x dilution factor/ Sample volume

Enrichment

10 g of the sample dissolved in buffered sodium chloride peptone was transferred to 90 mL of Tryptic Soy Broth (TSB). This medium contains lecithin and polysorbate required for neutralisation and is a general producer medium. After thorough shaking, the medium was incubated at 30-35°C for 18-24 hours (maximum 72 hours). After incubation, a selective medium was used. Enrichment was performed for *E. coli, P. aeruginosa and S. aureus*. For *C. albicans*, 10 mL (1 g or mL) of the sample dissolved in TSP was transferred to 90 mL Sabouraud Dextrose Broth (SDB). After shaking well, it was incubated at 30-35°C for 72 hours (maximum five days). After incubation, a selective medium was used.

Investigation of Aerobic Mesophilic Bacteria

After enrichment, 1 mL of TSB medium was taken and placed in sterile petri dishes. 5 mL of medium was added to Tryptone Glucose Extract Agar (TGEA) medium cooled to 45°C, mixed and inoculated with the sample in duplicate and allowed to solidify. After solidification, it was incubated at 37°C for 48 hours. In case of growth at the end of incubation, the number of colonies formed is calculated taking into account the dilution factor.

Investigation of The Presence of *Escherichia Coli*

After enrichment, 1 mL of TSB medium was taken and placed in sterile petri dishes. Then 5 ml of medium was added to Macconkey Agar (MCA) medium, mixed and the medium was inoculated with the sample in duplicate and allowed to solidify. Incubated at 30-35°C for 24 hours (maximum 48 hours).

Investigation for The Presence of *Staphylococcus Aureus*

After enrichment, 1 mL of TSB medium was taken and placed in sterile petri dishes. 5 ml of medium was added to Mannitol Salt Agar (MSA) medium cooled to 45°C, inoculated with the sample in duplicate and allowed to solidify. Incubated at 30-35°C for 24 hours (maximum 48 hours).

Investigation of The Presence of *Pseudomonas Aeruginosa*

After enrichment, 1 mL of TSB medium was taken and placed in sterile petri dishes. 5 mL of medium was added to Cetrimide Agar (CA) medium cooled to 45°C, mixed and the medium was inoculated with the sample in duplicate and allowed to solidify. Plates were incubated at 25˚C for 5 to 7 days.

RESULTS

Microbiological analyses

Table 2. Microbiological compliance results applied to the samples taken into the study

Purifying Oil Cleanser with %2 Postbiotics				
Analyze	**Result (cfu/g)**	**Limit for eye contour products and products for use in children under 3 years of age**	**Limit for other products**	**Evaluation**
Aerobic Mesophilic Colony Count	<1.0E+1	1.00E+02	1.00E+03	Suitable
P. aeruginosa	Not Detected	Must not be found	Must not be found	Suitable
Escherichia coli	Not Detected	Must not be found	Must not be found	Suitable
Candida albicans	Not Detected	Must not be found	Must not be found	Suitable
S. aureus	Not Detected	Must not be found	Must not be found	Suitable
Total Mould - Yeast Count	<1.0E+1	1.00E+02	1.00E+03	Suitable

Cell viability

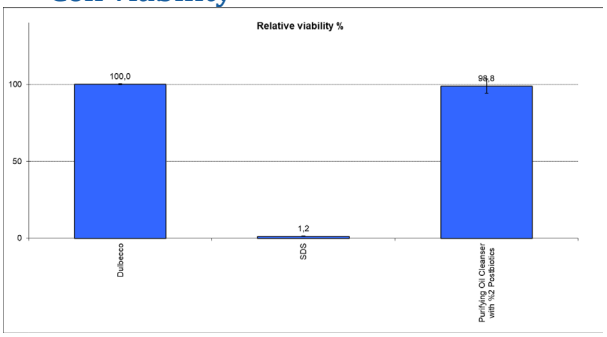

Figure 1. Cellular viability graphics.

According to the graphical data, the cleanser product containing 2% postbiotic did not show any cytotoxic effect on keratinocytes.

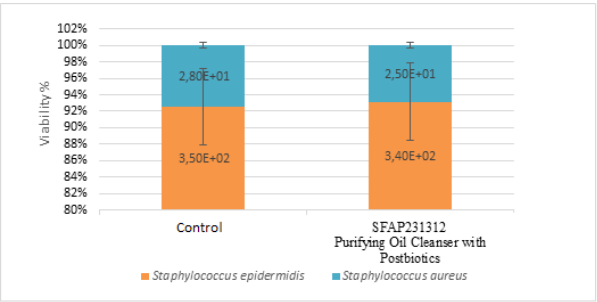

Figure 2. Pathogen non-pathogen balance graph of 2% postbiotic containing cleanser product in microbiota simulation test.

Microbiome & Microbiota Friendly Studies

It was hypothesised that the equilibrium test would demonstrate a preponderance of *S. epidermidis*. As this was observed, the study was continued. *S. epidermidis* is the indicator microorganism that suppresses the excessive increase of *S. aureus* species. The maintenance of a balanced microbiota is key to the suppression of infection. According to the graph, the tested product did not disturb the balance in favour of the pathogen.

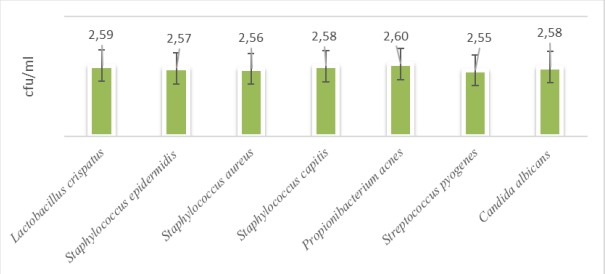

Figure 3. Plot of microbial diversity in the absence of samples.

The graph indicates that the initial diversity was determined in the analysis conducted without the addition of the tested product.

Table 3. Table of microbiological diversity results in absence of sample (control)

Microorganism	Control (PBS) cfu/ml	Control (PBS) log10
Lactobacillus crispatus	3.88E+02	2.59
Staphylococcus epidermidis	3.68E+02	2.57
Staphylococcus aureus	3.66E+02	2.56
Staphylococcus capitis	3.84E+02	2.58
Propionibacterium acnes	4.00E+02	2.60
Streptococcus pyogenes	3.55E+02	2.55
Candida albicans	3.78E+02	2.58

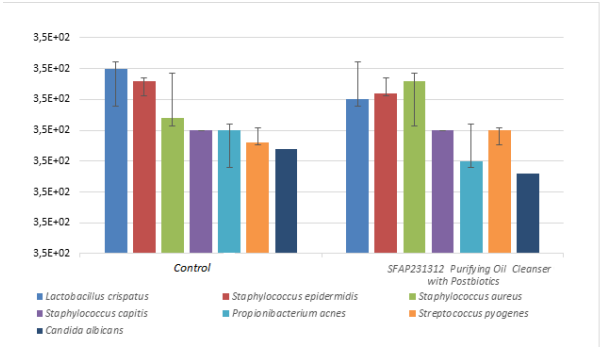

Figure 4. Microbial diversity graph of Purifying oil cleanser product containing 2% postbiotic.

The results of the study, as presented in the graph, indicate that the product containing 2% postbiotic did not result in any adverse effects on the diversity of microbiota.

Table 4. Table of microbiological diversity results in the presence of sample

Microorganism	Inoculum (cfu/ml)	Control (cfu/ml)	SFAP231312- Purifying Oil Cleanser with Postbiotics (cfu/ml)	Control (log10)	SFAP231312- Purifying Oil Cleanser with Postbiotics (log10)
Lactobacillus crispatus	3.50E+02	3.5E+02	3.5E+02	2.5	2.5
Staphylococcus epidermidis	3.50E+02	3.5E+02	3.5E+02	2.5	2.5
Staphylococcus aureus	3.50E+02	3.5E+02	3.5E+02	2.5	2.5
Staphylococcus capitis	3.50E+02	3.5E+02	3.5E+02	2.5	2.5
Propionibacterium acnes	3.50E+02	3.5E+02	3.5E+02	2.5	2.5
Streptococcus pyogenes	3.50E+02	3.5E+02	3.5E+02	2.5	2.5
Candida albicans	3.50E+02	3.5E+02	3.5E+02	2.5	2.5

The incorporation of the sample did not result in any impairment to the microbiological diversity. The study evaluated the change in microorganisms contacted with the sample in comparison to the control. The results demonstrated that the samples exhibited

no biocidal effect on microorganisms. The study's hypothesis was based on the principle that the difference between the initial amount and the amount after contact would not exceed 60%.

DISCUSSION

In recent years, there has been a marked shift in emphasis towards personalised medicine and preventive healthcare. This has resulted in a significant increase in consumer interest in natural-based cosmetics and personal care products. In the context of this paradigm shift, the importance of the skin microbiota is increasingly recognised due to its central role in mediating and regulating skin homeostasis (29). In conventional wisdom, the employment of live microorganisms has been a staple approach to promoting dermal health. However, the formulation and stability of these microorganisms can pose substantial challenges. Recent research has garnered mounting attention on the potential benefits of postbiotics, defined as metabolic byproducts secreted by microorganisms, particularly probiotic bacteria (30). Postbiotics are increasingly being recognised as promising ingredients for topical cosmetic formulations. They offer several advantages, including stability, safety and the ability to penetrate the skin barrier. Postbiotics have been shown to help maintain the body's homeostasis and aid in cellular repair. Furthermore, postbiotics have been demonstrated to enable microorganisms to function better (31). This research study investigates the safety and potential benefits of a new oil-based cleanser formulation, which contains a postbiotic in oil-soluble form as its unique feature. The results demonstrate that the formulation does not harm human keratinocytes and maintains a robust balance between pathogenic and commensal species in an in *vitro* skin microbiota simulation. Furthermore, cell viability tests show that the formulation does not cause significant adverse effects on the viability of human keratinocytes. This finding suggests that the formulation does not possess intrinsic toxicity, which is a significant indication in the field. It is particularly noteworthy that the majority of cosmetic products, notably cleansing formulations, are intended for long-term or frequent use. Therefore, it is imperative to preserve the integrity of keratinocytes, as elevated levels of toxicity can result in the disruption of skin barrier function, inflammation, and adverse reactions.In this context, the formulation appears to be safe for maintaining the health of epidermal cells.

The present study demonstrated that the ratio between non-pathogenic and pathogenic bacteria in an *in vitro* skin microbiota simulation, a community found in human skin, remained unaltered without compromising the balance. A healthy skin microbiota has been shown to assist in resisting pathogen colonization of human skin, modulating immune function, and maintaining the skin barrier. Numerous skin diseases are characterised by microbiota imbalance or overgrowth of certain pathogenic species, in contrast to the relative abundance associated with healthy microbiota.

The formulation that was the subject of this investigation has been shown to preserve microbial balance and to promote a healthy microbiota profile. This suggests that it has the potential to provide effective yet gentle cleansing without disrupting skin

barrier integrity or homeostasis.The benefits that were observed – specifically, the stimulation of beneficial species and suppression of pathogenic growth – are likely to be mediated by the inclusion of the postbiotic LTO 35, a metabolite derived from *Streptococcus thermophilus.* Postbiotics, defined as bioactive compounds produced by microbial fermentation (e.g., cell-free supernatant, enzymes, or organic acids), are increasingly recognised for their dermatological applications.In this context, the S. thermophilus-derived postbiotic contains a synergistic blend of metabolites, including lactic acid, bacteriocins, short-chain fatty acids (SCFAs), and hydrolytic enzymes, each contributing to skin health through distinct mechanisms: Lactic acid and organic acids lower cutaneous pH, creating an inhospitable environment for pathogens while enhancing ceramide synthesis, which reinforces stratum corneum cohesion. Bacteriocins exhibit dual functionality: (i) direct antimicrobial activity against pathogens (e.g., *Staphylococcus aureus*) and (ii) immunomodulatory effects via interactions with keratinocyte toll-like receptors. SCFAs (e.g., acetate, propionate) regulate epidermal differentiation and attenuate inflammatory cascades by modulating dendritic cell signalling (32).

The present findings are consistent with the evidence that postbiotic metabolites derived from probiotic strains, in particular lactic acid bacteria, exert pleiotropic benefits on cutaneous health without compromising commensal microbiota diversity (33). The formulation's selectivity – in other words, its capacity to inhibit pathogens while fostering symbionts – underscores its potential as a topical agent that is compatible with the microbiome.

CONCLUSION

The study demonstrated that the purifying oil cleanser, containing 2% postbiotic LTO 35, preserves the diversity of the skin microbiota, does not cause pathogenic dominance, does not damage keratinocyte cells and promotes cellular repair. Furthermore, microbiological balance tests revealed that the product has microbiome-friendly properties while protecting the skin barrier. These findings suggest that postbiotic-based formulations may play an effective role in protective dermocosmetic products.

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REFERENCES

1. Prescott SL, Larcombe DL, Logan AC, West C, Burks W, Caraballo L, et al. The skin microbiome: impact of modern environments on skin ecology, barrier integrity, and systemic immune programming. World Allergy Organ J. 2017;10:1-16.
2. Roy S, Dhaneshwar S. Role of prebiotics, probiotics, and synbiotics in management of inflammatory bowel disease: Current perspectives. World J Gastroenterol. 2023;29(14):2078.
3. Zhao X, Liu S, Li S, Jiang W, Wang J, Xiao J, et al. Unlocking the power of postbiotics: A revolutionary approach to nutrition for humans and animals. Cell Metab. 2024;36(4):725-44.
4. Aguilar-Toalá JE, Garcia-Varela R, Garcia HS, Mata-Haro V, González-Córdova AF, Vallejo-Cordoba B, et al. Postbiotics: An evolving term within the functional foods field. Trends Food Sci Technol. 2018;75:105-14.
5. Taverniti V, Guglielmetti S. The immunomodulatory properties of probiotic microorganisms beyond their viability (ghost probiotics: proposal of paraprobiotic concept). Genes Nutr. 2011;6(3):261-74.

6. Patel RM, Denning PW. Therapeutic use of prebiotics, probiotics, and postbiotics to prevent necrotizing enterocolitis: what is the current evidence?. Clin Perinatol. 2013;40(1):11-25.
7. Sionek B, Gantner M. Probiotics and Paraprobiotics—New Proposal for Functional Food. Appl Sci. 2025;15(1).
8. Oleskin AV, Shenderov BA. Microbial Communication and Microbiota-Host Interactivity: Neurophysiological, Biotechnological, and Biopolitical Implications. Hauppauge, NY: Nova Science Publishers; 2020.
9. Kothari D, Patel S, Kim SK. Probiotic supplements might not be universally effective and safe: A review. Biomed Pharmacother. 2019;111:537-47.
10. Aslan İ, Tarhan Çelebi L. Postbiotics Cosmetic Formulation: In Vitro Efficacy Studies on a Microbiome Friendly Antiperspirant. J Res Pharm. 2023;27(5).
11. Gökçe HB, Aslan İ. Novel Liposome–Gel Formulations Containing a Next Generation Postbiotic: Characterization, Rheological, Stability, Release Kinetic, and In Vitro Antimicrobial Activity Studies. Gels. 2024;10(11):746.
12. Bursalıoğlu E, Tarhan L, Çakıcı B, Genel N, Kalbişen HT, Aslan İ. Can buttermilk (ayran) with its postbiotic content be used in the treatment of colon cancer?. J Immunol Clin Microbiol. 2024;9(4):130-40.
13. Aslan İ, Kurt AA. Characterization and Optimization of Phytosome Formulation Containing Alcohol-free Umckalin from Pelargonium sidoides. Curr Perspect Med Aromat Plants. 2020;3(1):49-53.
14. Aslan İ, Kurt AA. In-vitro comparison release study of novel liposome and conventional formulation containing Rosmarinus officinalis extract. Curr Perspect Med Aromat Plants. 2021;4(1):13-21.
15. Kurt AA, Ibrahim B. Preparation of New Generation Natural Repellent Formulations From Essential Oils: From Basic Research to Technological Development. J Cosmet Sci. 2024;75(4).
16. Ibrahim B, Kurt AA. Mikrobiyolojik Olarak Test Edilmiş Bitkisel Ekstraktlar ve Esansiyel Yağlar ile Saç Dökülmesine Karşı Doğal Şampuan Formülasyon Geliştirilmesi. J Immunol Clin Microbiol. 2024;9(1):12-23.
17. Kurt AA, Ibrahim B. New generation natural face cream formulation: development and in vitro evaluation. Celal Bayar Univ Sag Bilim Enst Derg. 2024;11(4):672-80.
18. Kurt AA, Aslan I, Duman G. Next-Generation Natural Baby Barrier Cream Formulations: Physicochemical Analysis and Safety Assessment. J Cosmet Sci. 2021;72(2).
19. Bursalioğlu EO. Evaluation of antibacterial activity of Triticum monococcum seeds, Castanea sativa seeds and Begonia maculata leaves against several bacterial strains. Turk J Biodiv. 2020;3:9-14.
20. Buran K. Design, Synthesis, Biological Evaluation and Docking, ADME Studies of Novel Phenylsulfonyl Piperazine Analogues as α-Amylase Inhibitors. Cumhuriyet Sci J. 2024;45(2):268-73.
21. Buran K, İnan Y, Akyüz GS, Özdemir CD, Kocabas F. Phenylsulfonylpiperazines as α-Glucosidase Enzyme Inhibitors: Design, Synthesis, DFT Calculations, Docking and ADME Studies. Bitlis Eren Univ Fen Bilim Derg. 2024;13(3):723-30.
22. Buran K. Benzoin-tryptamine Schiff base-metal complexes: synthesis, DFT calculation and antimicrobial activities. J Mol Struct. 2025;141499.
23. Grice EA, Segre JA. The skin microbiome. Nat Rev Microbiol. 2011;9(4):244-53.
24. Barrandon Y, Green H. Three clonal types of keratinocyte with different capacities for multiplication. Proc Natl Acad Sci U S A. 1987;84(8):2302-6.
25. Schmidt-Ullrich R, Paus R. Molecular principles of hair follicle induction and morphogenesis. Bioessays. 2005;27(3):247-61.
26. Tickle C. Making digit patterns in the vertebrate limb. Nat Rev Mol Cell Biol. 2006;7(1):45-53.
27. Panati C. Panati's extraordinary origins of everyday things. Chartwell Books; 2016.
28. A framework for human microbiome research. Nature. 2012;486(7402):215-21.
29. Ajayi SA, Olaniyi OO, Oladoyinbo TO, Ajayi ND, Olaniyi FC. Sustainable Sourcing of Organic Skincare Ingredients: A Critical Analysis of Ethical Concerns and Environmental Implications. Asian J Adv Res Rep. 2024;18(1):65-91.
30. Prescott SL, Larcombe DL, Logan AC, West C, Burks W, et al. The skin microbiome: impact of modern environments on skin ecology, barrier integrity, and systemic immune programming. World Allergy Organ J. 2019;10:29.
31. Silva Vale A, Melo Pereira G, Oliveira AC, Carvalho Neto DP, et al. Production, Formulation, and Application of Postbiotics in the Treatment of Skin Conditions. Fermentation. 2023;9(3):264.
32. Sanford JA, Zhang LJ, Williams MR, Gangoiti JA, Huang CM, Gallo RL. Short-chain fatty acids from Cutibacterium acnes activate both a canonical and epigenetic inflammatory response in human sebocytes. J Immunol. 2019;202(6):1767-76.
33. Aslan I, Tarhan Celebi L, Kayhan H, Kizilay E, Gulbahar MY, Kurt H, et al. Probiotic formulations containing fixed and essential oils ameliorates SIBO-Induced gut dysbiosis in rats. Pharmaceuticals. 2023;16(7):1041.

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CASE REPORT/OLGU SUNUMU

Case report: Example of the impact of laboratory processes on patient safety: Reflection of 'HBsAg' test results on analytical and post-analytical process management

Laboratuvar süreçlerinin hasta güvenliğine etkisine örnek: 'HBsAg' test sonuçlarının analitik ve post-analitik süreç yönetimine yansıması

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Abstract

Hepatitis B virus (HBV) infections are a group of infections that primarily affect the liver and cause inflammatory hepatitis. This group of infections; In Turkey, it develops as acute hepatitis at a rate of 2% to 10%, and 4% to 6% of these cases later develop as chronic hepatitis. 10% of chronic hepatitis cases face the risk of developing hepatocellular carcinoma (HCC) in the future. Various methods are used in the diagnosis of HBV infections. The most commonly used method in diagnosis is the screening of serological indicators (ELISA tests: HBsAg, Anti-HBs, Anti-HBc IgM and Anti-HBc IgG) in serum. Among these indicators, the 'HBsAg' test is the first test used for screening purposes for HBV infection and in the study and interpretation of test results; the evaluation is made by taking into account various factors related to the patient, employee and test procedure. Accuracy and reliability of laboratory test results; it is a very important parameter in terms of both patient safety and control of laboratory test processes. In our case; based on the 'HBsAg' test result of one patient, our studies and recommendations regarding the false positive HBsAg test results detected in a total of three patients are included. In the root cause analysis studies conducted on the process of 3 patients with unusual 'HBsAg' positivity; lack of training among the relevant personnel, lack of awareness, failure to carry out device technical service maintenance checks on time, and deficiencies in communication with clinicians came to the fore. As a result of our work on these inappropriate test results; Regular service maintenance and checks of ELISA devices, follow-up and traceability of quality-control studies, and effective communication between the patient's physician and the patient's clinic will prevent the development of situations that may jeopardize patient safety on a laboratory basis.

Keywords: Patient safety, Hepatitis B virus (HBV), Test result

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Öz

Hepatit B virüs (HBV) enfeksiyonları, öncelikle karaciğeri etkileyen ve inflamatuvar hepatite neden olan bir grup enfeksiyondur. Bu grup enfeksiyonlar; Türkiye'de %2 ila %10 oranında akut hepatit olarak gelişmekte ve bu vakaların %4 ila %6'sı daha sonra kronik hepatit olarak gelişmektedir. Kronik hepatit vakalarının %10'u ileride hepatosellüler karsinom (HCC) gelişme riski ile karşı karşıyadır. HBV enfeksiyonlarının tanısında çeşitli yöntemler kullanılmaktadır. Tanıda en sık kullanılan yöntem serumda serolojik göstergelerin (ELISA testleri: HBsAg, Anti-HBs, Anti-HBc IgM ve Anti-HBc IgG) taranmasıdır. Bu göstergeler arasında 'HBsAg' testi HBV enfeksiyonu için tarama amaçlı kullanılan ilk testtir ve test sonuçlarının incelenmesi ve yorumlanmasında; hasta, çalışan ve test prosedürü ile ilgili çeşitli faktörler göz önünde bulundurularak değerlendirme yapılır. Laboratuvar test sonuçlarının doğruluğu ve güvenilirliği; hem hasta güvenliği hem de laboratuvar test süreçlerinin kontrolü açısından çok önemli bir parametredir. Olgumuzda; bir hastanın 'HBsAg' test sonucundan yola çıkarak toplam üç hastada tespit edilen yanlış pozitif HBsAg test sonuçlarına ilişkin çalışmalarımız ve önerilerimiz yer almaktadır. Olağandışı 'HBsAg' pozitifliği tespit edilen 3 hastanın sürecine ilişkin yapılan kök neden analizi çalışmalarında; ilgili personelin eğitim eksikliği, farkındalık eksikliği, cihaz teknik servis bakım kontrollerinin zamanında yapılmaması ve klinisyenlerle iletişimdeki eksiklikler ön plana çıkmıştır. Bu uygunsuz test sonuçları üzerine yaptığımız çalışma sonucunda; ELISA cihazlarının düzenli servis bakım ve kontrollerinin yapılması, kalite-kontrol çalışmalarının takibi ve izlenebilirliği, hastanın hekimi ve hastanın kliniği arasında etkin iletişimin sağlanması laboratuvar bazında hasta güvenliğini tehlikeye atabilecek durumların gelişmesini engelleyecektir.

Anahtar Kelimeler: Hasta güvenliği, Hepatit B virüsü (HBV), Test sonucu

INTRODUCTION

People can grouped who may be in the risk group in screening tests for HBV infections; healthcare workers, born in, living in, and migrating from medium-high endemicity regions (prevalence >2), living with HBV-infected people and with a history of sexual contact, IV (Intravenous) drug addicts and who constantly receive blood products, homosexuals, polygamists, heterosexuals and HIV(+) patient groups, with chronic liver disease (infected with HIV/HCV), hemodialysis patients, receiving immunosuppressive treatment, pregnant women and babies born to HBV-infected mothers (1).

Evaluation of 'HBsAg' tests and possible interference situations

Nowadays, for HBsAg tests, either manual (Immunochromatographic-Card test) or Enzyme immunoassay (EIA) methods are generally used. There are situations where both methods used have advantages and disadvantages compared to each other. In a study; while the sensitivity of the card test is low (93%) and the specificity rate is 100%; In EIA tests, the sensitivity rate was found to be high (100%) and the specificity rate was found to be 100% (2).

When evaluating HBsAg screening test results, there are various factors that may cause misinterpretation of the test results. Especially in cases where isolated HBsAg positivity is detected; the patient being in the initial (incubation) period of acute HBV, a history of blood transfusion from an HBsAg positive person, the presence of chronic HBV infection that does not develop an anti-HBc response, the use of test kits and the presence of problems related to the test device, sample-related contamination, antigenemia cases after high-dose hepatitis vaccine in young children and the presence

of HBV-S mutants should be evaluated (3).

Features of the 'HBsAg' kit that is used in the ELISA device in the laboratory

The test kit used for HBsAg screening is used in our laboratory with a single device and a single test kit; It is the Architect HBsAg Qualitative II test kit from Abbott company and performs the test procedure with chemiluminescent microparticle immunoassay technology. The HBsAg antigen detection range of the test (measurable range) is defined as ≥1 (Sample/Cut-off: S/CO), and in the kit package insert the this test kit specificity is stated as 99.91% and the sensitivity is 99.09%. In the kit package insert; The issues that need to be taken into consideration in the selection of test samples, storage of test kits and use of test reagents during the testing stages are also emphasized (Abbott, Architect system, HBsAg II Qualitative test prospectus) (4).

CASE PRESENTATION

As of September 18, 2023; since we have never encountered this situation before, in order to interpret it in line with the information request of the relevant patient and to examine this situation we decided to make an evaluation about 'HBsAg' test result approved on 'August 21, 2023' . On this date (August 21, 2023); The HBsAg test result of the patient (49 years old, female) was measured as 53.75 S/CO (positive) on the Abbott Architect device and was approved by us. The patient's biochemistry test results were normal and there were no abnormal laboratory findings. The same patient had the HBsAg test done again in Izmir, 1 week after August 21, 2023 (29.08.2023), and this time the test result was measured as '0.18' S/CO (negative). Upon this result, the patient said; She reported his complaint to the patient rights unit of our hospital, saying "They diagnosed me with Hepatitis B" and requested information from the laboratory. Upon this request, we examined the package insert of the HBsAg test kit used in the laboratory and conducted a literature search to examine the situations that may cause HBsAg cross-reactions (false positive results). At the end of both source research; we told to the patient rights unit to write an answer, explaining that may be cross-positive results due to situations which is arising from the patient status (hunger-satiety, vaccination history, non-HBV viral and bacterial hepatitis, autoimmune disease, immunosuppressive treatment, etc.) or test kit (thermostability of the kit, interaction due to serum content, gray zone due to test sensitivity). 2 days after September 18, 2023, on September 20, 2023, the HBsAg test result of another patient (70 years old, M) diagnosed with vitamin D deficiency, who entered the infection clinic, was 73.95 S/CO (positive) with the same device and kit. The test has been concluded and the test result has been approved by us. The next day (21.09.2023), the infectious disease doctor called laboratory and said that the patient did not have a risky condition in terms of HBV, that there could be that she would request a blood test for HBsAg from the same patient again. We suggested working with patient serum that gives results that was tested the day before and tested positive. As of September 21, 2023; with the same device and the same kit, the serum that gave a positive result the day before was '0.24' S/CO (negative) on the second day, and the test result of the patient's blood on the second day was '0.25' S/CO (negative). In a short period of time; Considering these results, it was thought that the situation was caused by the test or device, not the patient, and examinations related to the device and test kit were carried out to perform root-cause analysis.

The results of the calibration and internal quality and external quality control studies performed on the device on the days when the tests of the relevant patients were studied were appropriate and approved by us. For the ELISA device, which was installed in April 2023, the technical service has come for routine maintenance for the ELISA device several times since the device installation time (about 6 months), only for technical equipment repair and several times for %CV (consecutive work-test for analytical measurement error analysis-study on the

coefficient of variation) was carried out and there were records associated with regarding this, but there was no record regarding detailed (comprehensive) technical service, control and maintenance. There upon, a interwiewed was held with the relevant company officials on September 22, 2023, and they came from the relevant company technical service for detailed maintenance of the device on September 27, 2023. On the same day (September 27, 2023); Before the technical service maintenance work, the HBsAg test result of another 60-year-old male patient, who was referred to the laboratory from the internal medicine outpatient clinic with the diagnosis of 'vitamin D deficiency', was measured as '101.25' S/CO (positive) even though his other laboratory tests were normal. It was not approved, the physician who requested the tests was contacted, the patient's risk status in terms of HBV was questioned, and it was learned that there was no risky situation for the patient. After technical service control and maintenance; In this serum put back into the device; The result, which was previously measured as '101.25' S/CO, was this time measured as '0.19' (negative) S/CO, and it was stated to the relevant physician that such a result was encountered due to a problem related to the device and kit (Table 1).

Table 1. Evaluation of patients' HBsAg test results

Patients	1st test result (S/CO) and date	2nd test result (S/CO) and date	Comment
Patient 1	53,75 (21.08.2023)	0,18 (29.08.2023)	False positive result
Patient 2	73,95 (20.09.2023)	0,24 (21.09.2023)	False positive result
Patient 3	101,25 (27.09.2023)	0,19 (27.09.2023)	False positive result

CONCLUSION AND RECOMMENDATİONS

Medical microbiology laboratory processes consist of 5 parts; pre-preanalytical process, pre-analytical process, analytic process, post-analytical process and post-postanalytical process. Pre-preanalytical process; It covers processes such as informing physicians about the test guide, making test orders in accordance with the indications, applying decision-support systems, and designing test panels and test order forms. Pre-analytical process; It includes the processes of preparing the patient for sample collection, sample collection, sample transfer, acceptance of the sample to the laboratory and preparation for analysis. Analytical process; It is a process that includes the control of materials, devices and equipment used in performing tests in the laboratory, the competence of the personnel, device/ test quality control studies and validation studies, and consists of measurable and controllable parameters. Post-analytical process; It includes processes related to determining test result delivery times and informing the target audience about these times, arrangements for patient result reports and the design of the minimum information that should be included in these reports, and archiving of patient test results and related reports. Post-analytical process; It is the process that includes effective notification of test results (panic/ critical value) that will affect the safety of the patient to the relevant physician, information and guidance support for the interpretation of test results, and practices to encourage rational antibiotic use (5).

In order to avoid such risky situations or to minimize the possibility of encountering them, laboratory analytical and post-analytical process controls must be managed effectively and accurately. In the root cause analysis studies conducted on the process of 3 patients with unusual 'HBsAg' positivity; Lack of training among