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ORIGINAL RESEARCH

Body Mass Index and Waist-Hip Ratio as Health Risk Predictors among selected Southern Nigerian University Undergraduates

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Abstract

***Objective**: The use of clinical anthropometry in the evaluation of several forms of health risks associated with dietary patterns and lifestyle habits has been encouraged globally. This present study aimed to assess the body mass index (BMI) and waist-hip ratio (WHR) of selected South-Southern Nigerian tertiary students.*

***Methods**: The study involved 100 students (50 males: 50 females) of Madonna University, Elele between 18 to 25 years. The health risk classification based on the BMI of both genders was grouped into seven (7) classes; severely underweight, underweight, normal weight, overweight, obese I, II, and III. The WHR health risk classification was grouped into three (3) classes for both genders; low, moderate, and high risks. A stadiometer was calibrated to the nearest 0.01m to obtain body height while body weight was measured to the nearest 0.1kg with a HD358 Tanita digital bathroom weighing scale. Waist (WC) and hip circumferences (HC) were measured to the nearest 0.5cm with a non-stretchable measuring tape.*

***Results**: The study showed that the highest proportion of students was either normal (27%) or overweight (24%). Females had a mean BMI and WHR of 26.56kg/m^2 and 0.77, respectively while males had a mean BMI and WHR of 30.99kg/m^2 and 1.04, respectively. Based on WHR health risk classification, a higher percentage of males had a high risk (40%) in comparison to females (18%). There was a significant difference in the distribution of the WHR health risk classification between males and females (p=0.045).*

***Conclusion**: The current study concluded that both BMI and WHR could significantly be utilized as health risk predictors of disorders associated with diet and lifestyle habits in the study population.*

***Keywords**: Clinical anthropometry, body mass index, waist-hip ratio, South-Southern Nigeria, health risk.*

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Introduction

The assessment of health risks using anthropometric parameters involves using measurements of the human body, such as height, weight, body circumferences, and skinfold thickness, to evaluate an individual's body composition and potentially identify health-related concerns [1-3]. These measurements provide valuable insights into an individual's nutritional status, body fat distribution, and overall health risks. In the pursuit of achieving and maintaining optimal health, understanding and monitoring key indicators of body composition is crucial [4-5]. Two widely recognized metrics in this regard are Body Mass Index (BMI) and Waist-Hip Ratio (WHR). These measurements serve as valuable tools in assessing an individual's risk of various health conditions, ranging from cardiovascular diseases to metabolic disorders [3].

Body mass index (BMI) is a numerical value derived from an individual's weight and height [6]. It provides a standardized assessment of body composition, allowing for categorization into different weight status classifications such as underweight, normal weight, overweight, and obesity [6-8]. Underweight people usually have a BMI of less than 18.5, which is associated with health risks such as malnutrition, weakened immune system, osteoporosis, and reproductive issues [9-10]. Those with normal weight possess a BMI ranging from 18.5 to 24.9 and are generally considered a healthy range for most people. Overweight individuals tend to have a BMI between 25 to 29.9, which is closely linked to health risks including increased risk of heart disease, high blood pressure, type 2 diabetes, and other health issues. The Class I obese individuals do have a BMI ranging from 30 to 34.9 which predisposes them to certain health risks such as elevated risk of cardiovascular disease, type 2 diabetes, sleep apnea, and certain cancers [11-13]. Class II obese individuals have a BMI ranging from 35 to 39.9, accompanied by a significant increase in risk for serious health conditions including heart disease, stroke, and type-2 diabetes. Finally, class III obese individuals do have a BMI of 40 or higher, which together with an extremely high risk of developing serious health conditions like heart disease, stroke, diabetes, and certain cancers [11, 14]. Since its inception, BMI has been extensively utilized by healthcare professionals, researchers, and policymakers as an initial screening tool to evaluate an individual's weight-related health risks [6, 15-17].

In addition to BMI, the Waist-Hip ratio (WHR) offers a deeper insight into the distribution of body fat. The waist-hip ratio (WHR) is then calculated by dividing the waist circumference by the hip circumference. In men, the WHR classifications for health risks are as follows, low risk with a WHR less than 0.90, moderate risk with a WHR between 0.90 and 0.99, while for high risk, the WHR is usually 1.00 or higher. In women, the WHR classifications for health risks are as follows, low risk with a WHR less than 0.80, moderate risk with a WHR between 0.80 and 0.84, while for

high risk, the WHR is usually 0.85 or higher [18-20]. A lower WHR tends to suggest a healthier distribution of body fat, which is associated with a lower risk of cardiovascular diseases and other obesity-related health issues [11, 21-22]. Individuals who fall under the moderate WHR indicate an increased risk compared to individuals with a lower WHR. It suggests that there may be a higher proportion of abdominal fat, which is associated with higher health risks. A higher WHR indicates a potentially significant accumulation of abdominal fat. This is associated with a higher risk of conditions like heart disease, type 2 diabetes, and certain cancers [7, 23]. Unlike BMI, WHR specifically focuses on the distribution of fat around the abdomen and hips. This ratio is obtained by dividing the circumference of the waist by that of the hips. A higher WHR signifies an increased accumulation of visceral fat, which is known to be associated with a higher risk of metabolic disorders, cardiovascular diseases, and other health complications [24-26].

The significance of BMI and WHR lies not only in their ability to identify potential health risks but also in their versatility as tools for health interventions [7, 27]. By understanding the implications of these indicators, individuals can make informed decisions regarding lifestyle choices, including diet, exercise, and other preventive measures. Furthermore, healthcare providers can use these metrics to formulate personalized health plans and track the progress of their patients over time. For example, body mass index (BMI) is a commonly used tool for classifying individuals into different health risk categories based on their weight relative to their height. It provides a general assessment of whether a person is underweight, normal weight, overweight, or obese [6, 8]. The waist-hip ratio (WHR) is another useful tool for assessing health risks associated with body fat distribution. It takes into account the distribution of fat around the abdomen and hips as a higher WHR indicates that more fat around the waist is associated with increased health risks [11, 21, 28] . However, it is crucial to acknowledge that while BMI and WHR are valuable screening tools, they do have limitations. They do not provide a comprehensive assessment of an individual's overall health, taking into account factors such as muscle mass, bone density, and specific health conditions [29]. Though, a general approach to health assessment, involving additional measurements and clinical evaluations, is essential for a thorough understanding of an individual's well-being. Therefore, this current study aimed to evaluate the Body Mass Index (BMI) and Waist-Hip Ratio (WHR) of selected South-Southern Nigerian students and explore whether these variables can provide an understanding of possible health risks associated with these student populations.

Materials and Methods

This study employed a cross-sectional, and a descriptive design to assess the BMI and WHR of students of Madonna University, Elele within the ages of 18 to 25 years. Out of 100 students, 50 were males while 50 were females. Before the period of collection of data, ethical approval was obtained and informed consent was obtained from each participant as the purpose of this study was explained to them. The inclusion criteria were that selected participants were devoid of any physical deformities, and had not taken any meal six hours before the point of obtaining measurements. Individuals who met the inclusion criteria were randomly selected using a non-probability, convenience sampling technique. The materials used in carrying out the study include a stadiometer, measuring tape, and weighing scale.

Upon the calibration of the stadiometer to the nearest 0.01m, body height was measured while body weight was measured to the nearest 0.1kg with an HD358 Tanita digital bathroom weighing scale. The body mass index (BMI) was then calculated from the weight and height using the standardized formula ($\mathrm{BMI} = \mathrm{Weight}/\mathrm{Height}^2$). Both waist (WC) and hip circumferences (HC) were measured (in centimeters) to the nearest 0.5cm with a non-stretchable measuring tape. WC was measured at a point midway between the iliac crest and the lower rib margin on both sides while HC was measured at the widest point of the buttocks [30]. Waist-hip ratio (WHR) was calculated by dividing the waist circumference by the hip circumference. All measurements were performed twice and the calculated means were recorded to ensure the precision of data.

Statistical Analysis: Data was analyzed using Statistical Package for the Social Sciences (SPSS IBM version 23.0) and Microsoft Excel 2016 edition. Both descriptive and inferential statistics were employed and the results were presented in the form of tables. Independent sample t-test was used to determine gender differences in measured parameters. Pearson correlation was used to determine correlation statistics between observed variables in the studied population. The chi-square test was used to determine the association between gender and body mass index classification in the studied population. The levels of statistical significance were set at a p-value less than 0.05.

Results

Out of a total of 100 subjects as shown in Table 1, 5% were severely underweight, 3% were underweight, 27% had a normal weight, 24% were overweight, 19% belonged to the obese class I, 13% belonged to the obese class II and 9% belonged to the obese class III. Table 2 shows the descriptive statistics of weight, height, waist circumference (WC), hip circumference (HC), body mass index (BMI), and waist-hip ratio (WHR) in the population. The mean weight was 90.36kg,

mean height was 1.78m, mean WC was 77.96cm, mean HC was 89.16cm, mean BMI was 28.78kg/m^2, and mean WHR was 0.91.

Table 3 depicts the descriptive statistics of all variables for the female category. Their mean weight was 80.78kg, mean height was 1.75m, the mean WC was 72.13cm, mean HC was 94.22cm, mean BMI was 26.56kg/m^2, and the mean WHR was 0.77. Table 4 illustrates the descriptive statistics of all variables for the male category. Their mean weight was 99.94kg, mean height was 1.81m, the mean WC was 83.79cm, mean HC was 84.11cm, mean BMI was 30.99kg/m^2, and the mean WHR was 1.04.

Table 4 shows that using an independent t-test, the results showed male subjects had significantly higher mean values in weight, height, waist circumference (WC), body mass index (BMI), and waist-hip ratio (WHR) while females showed significantly higher mean value for hip circumference ($p<0.05$).

Table 5 describes the gender-based chi-square test of association in health risk classification using the BMI between male and female subjects in the studied population. No statistically significant difference was observed in the distribution of the health risk classification between males and females (p=0.052).

In Table 6, significant and strong correlations were observed between weight and waist circumference (r=0.580, p=0.001), and body mass index (r=0.786; p=0.001) while although significant, several weak correlations were observed between weight and height (r=0.263; p=0.008), hip circumference (r=0.207; p=0.039) and waist to hip ratio (r=0.298; p=0.003). A significant but weak correlation was observed between height and body mass index (r=-0.374; p=0.001). While weak and non-significant correlation was observed between height and waist circumference (r=0.102; p=0.314), hip circumference (r=0.001; p=0.998), and waist-to-hip ratio (r=0.089; p=0.376).

A significant but weak correlation was observed between waist circumference and hip circumference (r=0.433; p=0.001), body mass index (r=0.492; p=0.001), and waist-to-hip ratio (r=0.479; p=0.001). A significant but weak correlation was observed between hip circumference and body mass index (r=0.197; p=0.049) and waist-to-hip ratio (r=-0.551; p=0.001). A significant but weak correlation was also observed between body mass index and waist-to-hip ratio (r=0.235; p=0.018).

Table 7 explains the gender-based chi-square test of association in health risk classification using the WHR between male and female subjects in the studied population. Females with a lower health risk were more predominant than males with 70% and males with a higher health risk were more

predominant than the females with 40%. A statistically significant difference was observed in the distribution of the health risk classification between males and females (p=0.045).

Table 1. Distribution of Body Mass Index classification of the studied population

Body Mass Index classification	Frequency (%)
Severely underweight	5 (5.0)
Underweight	3 (3.0)
Normal	27 (27.0)
Overweight	24 (24.0)
Obese Class I	19 (19.0)
Obese Class II	13 (13.0)
Obese Class III	9 (9.0)

Table 2. Descriptive statistics of measured variables in the studied population.

Variables	N	Minimum	Maximum	Mean	Standard Deviation
Weight (kg)	100	43.00	140.00	90.36	22.89
Height (m)	100	1.37	2.08	1.78	0.15
WC (cm)	100	23.50	125.00	77.96	18.14
HC (cm)	100	24.50	121.30	89.16	20.84
BMI (kg/m^2)	100	13.99	45.82	28.78	7.72
WHR	100	0.51	2.05	0.91	0.28

N = Number of participants

Table 3. Descriptive statistics of measured variables based on gender in the studied population.

Gender	Variables	N	Minimum	Maximum	Mean	Standard Deviation
Female	Weight (kg)	50	43.00	140.00	80.78	25.02
	Height (m)	50	1.37	2.08	1.75	0.15
	WC (cm)	50	23.50	99.10	72.13	16.82
	HC (cm)	50	24.50	121.30	94.22	20.83
	BMI (kg/m^2)	50	13.99	45.82	26.56	8.23
	WHR	50	0.51	1.41	0.77	0.13
Male	Weight (kg)	50	73.00	140.00	99.94	15.67
	Height (m)	50	1.54	2.03	1.81	0.14
	WC (cm)	50	53.00	125.00	83.79	17.68

HC (cm)	50	50.80	115.70	84.11	19.79
BMI (kg/m^2)	50	20.11	44.65	30.99	6.55
WHR	50	0.59	2.05	1.04	0.32

N = Number of participants

Table 4. T-test inferential statistics of the observed variables based on gender in the studied population.

Variables	Gender	N	Mean	Standard Deviation	t	df	P-value
Weight (Kg)	Female	50	80.78	25.020	-4.589	98	**0.001**
	Male	50	99.94	15.672			
Height (m)	Female	50	1.7464	0.14679	-2.049	98	**0.043**
	Male	50	1.8060	0.14404			
WC (cm)	Female	50	72.1342	16.81653	-3.378	98	**0.001**
	Male	50	83.7886	17.67845			
HC (cm)	Female	50	94.2150	20.82454	2.488	98	**0.015**
	Male	50	84.1068	19.78919			
BMI (kg/m^2)	Female	50	26.5642	8.22490	-2.979	98	**0.004**
	Male	50	30.9920	6.54558			
WHR	Female	50	0.7696	0.13189	-0.562	98	**0.001**
	Male	50	1.0394	0.31661			

N = Number of participants, df = degree of freedom

Table 5. Gender-based Chi-square test of association in the BMI health risk classification in the studied population

Body Mass Index classification	Gender		Chi-square	df	p-value
	Female	Male			
Severely underweight	5 (10.0%)	-	12.475	6	0.052 (NS)
Underweight	3 (6.0%)	-			
Normal	16 (32.0%)	11 (22.0%)			
Overweight	11 (22.0%)	13 (26.0%)			
Obese Class I	6 (12.0%)	13 (26.0%)			
Obese Class II	5 (10.0%)	8 (16.0%)			
Obese Class III	4 (8.0%)	5 (10%.0)			

(NS = Not Significant)

Table 6. Pearson Correlation statistics between observed variables in the studied population.

		Weight (Kg)	Height (m)	WC (cm)	HC (cm)	BMI (kg/m^2)	WHR
Weight (Kg)	Pearson Correlation	1	0.263**	0.580**	0.207*	0.786**	0.298**
	Sig. (2-tailed)		**0.008**	**0.001**	**0.039**	**0.001**	**0.003**
	N	100	100	100	100	100	100
Height (m)	Pearson Correlation	0.263**	1	0.102	0.001	0.374**	0.089
	Sig. (2-tailed)	**0.008**		0.314	0.998	**0.001**	0.376
	N	100	100	100	100	100	100
WC (cm)	Pearson Correlation	0.580**	0.102	1	0.433**	0.492**	0.479**
	Sig. (2-tailed)	**0.001**	0.314		**0.001**	**0.001**	**0.001**
	N	100	100	100	100	100	100
HC (cm)	Pearson Correlation	0.207*	0.001	0.433**	1	0.197*	0.551**
	Sig. (2-tailed)	**0.039**	0.998	**0.001**		**0.049**	**0.001**
	N	100	100	100	100	100	100
BMI (kg/m^2)	Pearson Correlation	0.786**	-0.374**	0.492**	0.197*	1	0.235*
	Sig. (2-tailed)	**0.001**	**0.001**	**0.001**	**0.049**		**0.018**
	N	100	100	100	100	100	100
WHR	Pearson Correlation	0.298**	0.089	0.479**	-0.551**	0.235*	1
	Sig. (2-tailed)	**0.003**	0.376	**0.001**	**0.001**	**0.018**	
	N	100	100	100	100	100	100

**Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed).

Table 7. Gender-based Chi-square test of association in the WHR health risk classification in the studied population

Health Risk Classification	Gender		Chi-square	df	p-value
	Female	Male			
Low (Female: <0.80; Male <0.90)	35 (70.0%)	27 (54.0%)	6.205	2	**0.045**
Moderate (Female: 0.80 – 0.84; Male: 0.90 – 0.99)	6 (12.0%)	3 (6.0%)			
High (Female: ≥0.85; Male: ≥1.00)	9 (18.0%)	20 (40.0%)			

Discussions

The burden of health risks due to diet and lifestyle habits is a significant global public health concern [31-33]. Poor diet and unhealthy lifestyle choices contribute to a range of chronic diseases and conditions that can lead to reduced quality of life, increased healthcare costs, and even premature death [34-35]. Examples of the major health risks associated with diet and lifestyle habits include obesity, cardiovascular diseases, type 2 diabetes, hypertension, etc. The clinical assessments of health risks using anthropometric approaches involve the systematic measurement of various body parameters to gather information about an individual's physical health, growth, development, and nutritional status. These measurements are used to diagnose, monitor, and manage various health conditions [36-38]. They can provide awareness of an individual's body composition, including measures of body fat percentage, muscle mass, and distribution of fat. Parameters like weight-for-age, height-for-age, weight-for-height, and body mass index (BMI) are used to identify malnutrition, both under-nutrition and over-nutrition [39-40]. For individuals with chronic health conditions, such as obesity, diabetes, or cardiovascular disease, clinical anthropometry can be a valuable tool in assessing disease progression and response to treatment. The current study was done to understand the possible health risks of selected South-Southern Nigerian students upon the application of BMI and WHR.

Based on the assessment of health risks from BMI in the sample population of the present study, those with normal and overweight BMI formed the highest proportions among these undergraduates 27% and 24% respectively while other categories of BMI made up for the lowest proportions among them. The statement is consistent with global trends in BMI distribution, where individuals falling within the normal and overweight categories tend to be the majority in many populations. Research indicates that individuals in these categories generally have a lower risk of various health

conditions compared to those classified as underweight or obese [6, 41-42]. However, it's important to note that within the overweight category, there can still be varying levels of health risk depending on factors such as the distribution of fat and overall lifestyle. It is also important to recognize that BMI distributions can vary based on demographic factors such as age, gender, and ethnicity [43-44]. Furthermore, regional or cultural differences may influence the prevalence of different BMI categories [45-46]. While having a normal or overweight BMI might indicate a lower immediate risk of health problems, it does not necessarily guarantee long-term health. Lifestyle choices, such as diet and exercise, play a significant role in overall health and should be considered in conjunction with BMI.

In trying to explain and compare the descriptive statistics of measured variables between gender categories in line with related literature, there were significant differences between females and males in terms of weight, height, waist circumference (WC), hip circumference (HC), BMI, and waist-to-hip ratio (WHR). The mean BMI values for both females (26.56 kg/m²) and males (30.99 kg/m²) suggest that, on average, individuals in both categories fall within the overweight range. This is consistent with global trends, where a considerable portion of the population, regardless of gender, is classified as overweight or obese [6, 42, 46]. The mean waist circumference values for females (72.13 cm) and males (83.79 cm) indicate that, on average, the males have a larger waist circumference. Elevated waist circumference is associated with an increased risk of metabolic conditions like diabetes and cardiovascular diseases, as supported by research emphasizing the importance of central obesity as a predictor of health risks [24, 47]. The mean hip circumference values for females (94.22 cm) and males (84.11 cm) suggest that, on average, females have wider hips. The mean WHR for females (0.77) indicates a lower risk for central obesity-related health issues, while the male category's mean WHR (1.04) suggests a higher risk. This aligns with literature highlighting WHR as a valuable indicator of body fat distribution and associated health risks.

In line with the results of the present study, there was a significant and moderately strong positive correlation (r=0.580) between weight and waist circumference (WC). This suggests that as weight increases, WC tends to increase as well [6, 48]. Also, there was a significant and strong positive correlation (r=0.786) between weight and BMI. This means that as weight increases, BMI also tends to increase [6, 49-50]. However, there was a significant but relatively weak positive correlation (r=0.263) between weight and height, signifying that as weight increases, height tends to increase to some extent. Furthermore, there was a significant but weak positive correlation (r=0.207) between weight and hip circumference (HC), implying that as weight increases, HC tends to increase, but the relationship is not as strong as with WC [51]. The p-value of 0.039 indicates that this correlation is

statistically significant. In addition, there is a significant but weak positive correlation (r=0.298) between weight and waist-to-hip ratio (WHR). This means that as weight increases, the ratio tends to increase, indicating a potentially higher proportion of abdominal fat [6, 52-53]. The p-value of 0.003 indicates that this correlation is statistically significant. There is a significant but weak negative correlation (r=-0.374) between height and BMI. This suggests that as height increases, BMI tends to decrease.

As shown from the study results, there were significant differences in health risk classifications between males and females in the studied population when the WHR was considered. This means that a larger proportion of females (70%) in the study population were classified as having a lower health risk. This could be due to various factors such as differences in body composition, hormonal influences, and potentially healthier lifestyle habits. Conversely, among males, a larger percentage (40%) was classified as having a higher health risk. This could indicate that, on average, males in the study population had a higher proportion of risk factors associated with health conditions like obesity, heart disease, and diabetes [6, 54-55]. This indicates that the observed difference in health risk distribution between males and females is unlikely to have occurred by chance alone at a p-value of 0.045. The study suggests that there are notable gender differences in health risk distribution [56]. This highlights the importance of considering gender-specific health interventions and tailored approaches to address specific risk factors.

The limitations of this study would be that due to ethnic differences in body composition and genetics among racial populations, the health risks that are associated with the limits of BMIs and WHRs in this study might not be universally appropriate across other racial groups. The sample size of the study is only a true representation of the undergraduates of Madonna University, Elele, and not the entire undergraduate population of all Nigerian universities. **Conclusion**

The current study concludes that both BMI and WHR could significantly be utilized as health risk predictors of disorders associated with diet and lifestyle habits in the study population. Understanding the gender differences in the application of these clinical anthropometric variables can be crucial for public health agencies in South-Southern Nigeria towards the promotion of healthy lifestyles and management of health disorders associated with abuse of dietary and lifestyle habits.

These study findings might have implications for public health initiatives on the university campus. For example, there might be a need for targeted interventions to address issues related to nutrition and physical activity. It is also important to consider that while BMI is a useful screening tool, it

does not provide a complete picture of an individual's health. Other factors like muscle mass, body composition, and metabolic health should also be taken into account.

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Conflicts of Interest

There is no form of competing interests that exists among the authors.

Author's Contributions

All authors contributed to the various components of the study such as research design, collection of data and its analysis, write-up of the initial and final manuscript, and the submission of the finalized manuscript

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ORIGINAL RESEARCH

Antibiotic Susceptibility Patterns of Gram-Negative Anaerobic Bacteria Isolated from Clinical Samples

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Abstract:

Aim: *Although anaerobic bacteria are the normal flora of the body, they can cause infection with the weakening of the immune system. Isolation and identification of these bacteria are difficult and not performed in most laboratories. For this reason, anaerobic bacteria can often be ignored. As a result, antibiotic resistance situations that develop cannot be detected. We carried out this study to reveal that anaerobic bacteria are important, that knowing antibiotic resistance profiles is necessary for the course of treatment, and that antibiotic susceptibility tests should be performed at certain periods.*

Method: *This study included 372 samples sent from various clinical units to the Bacteriology/Culture Laboratory of Dicle University Faculty of Medicine Faculty of Medicine Department of Medical Microbiology. These samples were first inoculated on Brucella blood agar for bacteria isolation. After identifying the isolated bacteria with MALDI-TOF MS, the E-test method was used to determine the antimicrobial susceptibility profiles.*

Results: *Antibiotics with the highest antimicrobial resistance were determined as 100% penicillin (P), 41.2% clindamycin (CM) and 35.3% amoxicillin-clavulanic acid (AMC), respectively in 17 isolated Bacterioides species. Antibiotics with the highest antimicrobial resistance in the 22 isolated Prevotella species were determined as 45.5% penicillin (P) and 27.3% moxifloxacin (MX). Again, only metronidazole (MZ) resistance was observed in 1 Dialister pneumosintes bacteria, whereas resistance was observed against penicillin (P), imipenem (IP) and piperacillin-tazobactam (TPZ) antibiotics in 1 Veillonella parvula bacteria. Finally, resistance was observed against any antibiotic in 4 Fusobacterium species isolated.*

Conclusion: *It was observed that the majority of the Gram-negative anaerobic bacteria isolated in our study developed resistance to penicillin, clindamycin, moxifloxacin, metronidazole and amoxicillin-clavulanic acid, or had an increased resistance to these antibiotics, and were also sensitive to the remaining antibiotics.*

Key Words: *Gram-Negative Anaerobic Bacteria, Antibiotic Sensitivity, MIC, MALDI-TOF MS*

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Özet:

Amaç: *Anaerop bakteriler vücudun normal flora elemanı olmasına karşın, bağışıklık sisteminin zayıflamasıyla enfeksiyona neden olabilirler. Bu bakterilerin izolasyonları ve identifikasyonları güç olduğu için çoğu laboratuvarda yapılmamaktadır. Bu sebeple çoğu zaman anaerop bakteriler göz ardı edilebilmektedir. Bunun sonucu olarak da gelişen antibiyotik direnç durumları tespit edilememektedir. Çalışmamız anaerop bakterilerin antibiyotik direnç profillerinin bilinmesinin tedavinin seyri açısından gerekli olduğunu ve belirli periyodlarda antibiyotik duyarlılık testlerinin yapılması gerektiğini ortaya koymaktır.*

Metod: *Çalışmaya, Dicle Üniversitesi Tıp Fakültesi Tıbbi Mikrobiyoloji AD. Bakteriyoloji/Kültür laboratuvarına, çeşitli klinik birimlerden anaerop kültür istemiyle gönderilmiş 372 numune dahil edilmiştir. Bu numuneler, ilk olarak bakteri izolasyonu için Brucella kanlı agara ekildi. İzole edilen bakterilerin identifikasyonları MALDI-TOF MS ile yapıldıktan sonra antimikrobiyal duyarlılık profillerinin belirlenmesi için E – test yöntemi kullanıldı.*

Bulgular: *İzole edilen 17 Bacterioides türünde antimikrobiyal direncin en yüksek olduğu antibiyotikler sırasıyla % 100 oranında penisilin (P), % 41,2 oranında klindamisin (CM) ve % 35,3 oranında ise amoksisillin-klavulanik asid (AMC) olarak belirlendi. İzole edilen 22 Prevotella türünde ise antimikrobiyal direncin en yüksek olduğu antibiyotikler % 45,5 oranında penisilin (P) ve % 27,3 oranında moksifloksasin (MX) olarak belirlendi. Yine izole edilen 1 Dialister pneumosintes bakterisinde sadece metronidazol (MZ) direnci gözlemlenirken, 1 Veillonella parvula bakterisinde ise penisilin (P), imipenem (IP) ve piperasillin-tazobaktam (TPZ) antibiyotiklerine karşı direnç gözlemlenmiştir. Son olarak da izole edilen 4 Fusobacterium türünde ise hiçbir antibiyotiğe karşı direnç gözlemlenmemiştir.*

Sonuç: *Çalışmamızda izole edilen Gram-negatif anaerop bakterilerin büyük çoğunluğunun penisilin, klindamisin, moksifloksasin, metronidazol ve amoksisillin-klavulanik aside karşı bir direnç geliştirdiği veya bu antibiyotiklere karşı artan bir dirence sahip olduğu görülmüştür.*

Anahtar Sözcükler: *Gram-Negatif Anaerop Bakteriler, Antibiyotik Duyarlılığı, MIC, MALDI-TOF MS*

Introduction

For a long time, humanity has thought that oxygen is absolutely necessary for living things to maintain their vitality. As a result of the studies carried out by scientists, especially since the 18th century, they have shown that oxygen is not absolutely necessary for some microorganisms, or that these microorganisms can continue their vitality even if it is at very low levels. On the contrary, it has been demonstrated that oxygen has a toxic effect on these microorganisms.[1–4] Towards the end of the 19th century, Louis Pasteur, one of the founders of modern microbiology, divided microorganisms into 'aerobic' and 'anaerobic' according to their oxygen needs.[5–7]

In recent times, there has been a notable rise in global antimicrobial resistance, posing a heightened risk of severe clinical outcomes and mortality. [8] Despite numerous global studies on the resistance of anaerobic bacteria to antimicrobial agents [9], the existing data are incomplete due to technical challenges associated with isolating bacteria and conducting susceptibility testing. This process is not only expensive and time-consuming but also demands expertise. Furthermore, the absence of

universally applied guidelines for susceptibility testing and interpretation criteria adds to the challenge of accurately assessing the resistance of anaerobic bacteria to eradication therapy.[10]

The widespread antibiotic resistance seen recently in anaerobic bacteria seriously delays treatment, especially in polymicrobial infections. For this reason, it becomes important to identify isolated anaerobic bacteria and perform antibiotic sensitivity tests. However, since this process is laborious and time-consuming, it is recommended to be done in some special cases.[11] First of all, in anaerobic infections, surgical procedures such as abscess drainage, debridement of necrotic tissues, surface antisepsis, contact of the infection area with oxygen, elimination of foreign bodies and removal of obstructions should be performed. Antibiotics should be used as a continuation of treatment. Since bacterial isolation and identification in anaerobic infections is time-consuming and troublesome, patient treatment primarily begins with empirical treatment. For this reason, antibiotic susceptibility results of identified anaerobic bacteria are important for empirical treatment. The high antibiotic resistance in anaerobic bacteria seen recently requires antibiotic resistance monitoring at certain periods, especially for the success of empirical treatment. Therefore, reporting the antibiotic susceptibility results of anaerobic bacteria provides guidance in the treatment of serious infections such as endocarditis, recurrent and resistant bacteremia, lung abscess, pulmonary hypertension, osteomyelitis, which require long-term treatment, or in the treatment of infections that do not respond to empirical treatment. While sensitivity to commonly used antibiotics such as nitroimidazoles, carbapenems, chloramphenicol, beta lactams combined with beta lactamase inhibitors, penicillin, cefoxitin and clindamycin is gradually decreasing, resistance genes against these antibiotics have also been identified. These resistance genes can be carried between bacteria via genetic material transfer mechanisms.

Our aim in this study is to demonstrate that determining the antibiotic resistance profiles of anaerobic bacteria is necessary for the course of treatment and that antibiotic sensitivity tests should be performed at certain periods.

Materials and Methods

Collection of Samples

This study included 372 samples sent from various clinical units to the Bacteriology/Culture Laboratory of Dicle University Faculty of Medicine Faculty of Medicine Department of Medical Microbiology. Within this set of samples, we included one urine sample obtained through suprapubic aspiration and two tissue/abscess samples collected from the genital area through surgical procedures in the category of genital system samples. Additionally, two pleural fluid

samples obtained under sterile conditions were categorized as respiratory system samples and included in our study. The samples were delivered to the laboratory in anaerobic transport media and thioglycollate liquid media. Cultivation, purification and identification of all samples arriving at the laboratory were carried out without wasting time. All identified Gram-negative anaerobic bacteria were stored at – 80 °C in skim milk broth until the time of the study.

Cultivation of Samples

All suitable samples were cultured on brucella blood agar and thioglycollate liquid media. Thioglycollate broth used as return fluid was incubated at room temperature, and brucella blood agar plates were incubated in an anaerobic environment at 35-37°C for 24-72 hours. The morphological appearance and pigment formation of all colonies that appeared to grow at the end of incubation were evaluated and passaged for purification. The passaged plates were incubated again in an anaerobic environment at the appropriate temperature and time, and the identification phase began.

Identification of Isolated Anaerobic Bacteria

Plates were evaluated after 24-72 hours of incubation. Different techniques, devices, and materials were utilized to create an anaerobic culture environment. Foremost among these are the Anoxomat Jar Systems. In this system, where the anaerobic environment is automatically provided and controlled, an anaerobic atmosphere was established in jars connected to the system, containing anaerobic plates, with a gas mixture of 80-90% N_2, 5-10% CO_2, and 5% H_2. In addition to this, conventional methods such as anaerobic sealed bags, 2,5-liter anaerobic jars/jars, and standard incubation container systems were also employed. The incubation period of plaques without growth was extended to 5 days when necessary. Species-level identification of all different colonies growing in anaerobic plates was made with the MALDI-TOF MS. All anaerobic bacteria identified at species level were inoculated onto brucella blood agar. If the MALDI-TOF MS bacterial identification result scores were below the minimum species identification score (2,000 = min - 3,000 = max), the identification procedures were repeated. After the incubation period, 1-2 bacterial colonies taken from the passaged plates were transferred to the MALDI-TOF MS 96 sample working plate, dried, and 1µl of 70% formic acid was dropped on it and the steel plate was left to dry again. Finally, 1µl of matrix solution was dropped onto the steel plate, allowed to dry, and loaded into the device.

Determination of Antimicrobial Resistance of Isolated Anaerobic Bacteria

The E-test method was used to determine the antimicrobial resistance of Gram-negative anaerobic bacteria, purified and identified at species level, to 9 antibiotics. According to the European

Committee on Antimicrobial Susceptibility Testing (EUCAST Version 10.0: 2020) antibiotic susceptibility testing guide, 8 antibiotics (P, AMC, TPZ, IP, MP, CM, MZ, CL), Clinical Laboratory and Standard Institute (CLSI M100-ED30: 2020).) MIC values of 1 antibiotic (MX) were determined according to antibiotic susceptibility testing guidelines. The McFarland turbidity standard value of the purified and isolated Gram-negative anaerobic bacteria was set as 1.00. All bacterial suspensions, with the McFarland standard value set as 1.00, were inoculated onto the brucella blood agar surface with a 120° rotation at least three times with the help of a sterile swab. Before using the E-test strips, they were removed from –20 °C and allowed to reach 25 °C. With the help of sterile forceps, the strips were carefully placed on the agar surface, parallel and opposite to each other. A total of 9 antibiotic strips and 5 brucella blood agar plates were used for each bacterium. This process was done separately for each bacteria and was completed within a maximum of 15 minutes. Subsequently, the plates were transferred to an anaerobic environment and incubated at 37 ° C for 24-72 hours. As a result of incubation, the plates were evaluated and the point where the formed elliptical inhibition zone intersected with the antibiotic strip was accepted as the MIC value for that antibiotic. Thus, the determined MIC values were interpreted according to reference guidelines and it was determined whether the bacteria had antibiotic resistance.

Ethical Procedures

The Project titled as " Antibiotic susceptibility patterns of gram-negative anaerobic bacteria isolated from clinical specimen" planned by Selahattin ATMACA, Nida ÖZCAN and Alican BILDEN has been approved by the Ethics Committee of Dicle University Faculty of Medicine.

Results

This study included 372 samples sent from various clinical units to the Bacteriology/Culture Laboratory of Dicle University Faculty of Medicine Faculty of Medicine Department of Medical Microbiology. The samples were sent to the bacteriology laboratory. While 188 (50.5%) of 372 patients were female and 184 (49.5%) were male, the average age of these patients was 37 and the age range was 0 - 90. In addition, 260 (69.8%) of the samples were abscess, 95 (25.5%) were tissue/wound, 6 (1.6%) were blood, 5 (1.3%) were joint fluid, 4 (1)% were pleural/peritoneal fluid and 2 were urine. Of the 260 abscess samples, 33 (12.6%) were Gram-negative anaerobes, 15 (5.7%) were Gram-positive anaerobes, 2 (0.7%) were ARB (Acide Resistant Bacilli), 83 (9%). Facultative aerobic/anaerobic bacteria were 83 (% 31,9) of them. There was no growth in 127 (48.8%). Of the 95 tissue/wound samples, 7 (7.3%) grew Gram-negative anaerobic, 2 (2.1%) grew Gram-positive anaerobic, and 33 (34.7%) grew facultative aerobic/anaerobic bacteria. There was no

growth in 53 (55.7%) cases. Gram-positive anaerobic bacteria grew in 4 (66.6%) of 6 blood samples, and facultative aerobic/anaerobic bacteria grew in 1 (16.6%). There was no growth in 1 (16.6%). Of the 5 synovial fluid samples, 1 (20%) grew Gram-positive anaerobic bacteria and 2 (40%) grew facultative aerobic/anaerobic bacteria. There was no growth in 2 (40%) cases. Only 1 (25%) of 4 pleural/peritoneal fluid samples grew facultative aerobic/anaerobic bacteria. There was no growth in 3 (75%) cases. Gram-positive anaerobes grew in 1 of 2 urine samples (50%). There was no growth in 1 (50%). Skin, soft tissue and respiratory tract infection rates were lowest, and *Veillonella parvula* and *Dialister pneumosintes* were the least isolated species. Details of bacterial distribution according to isolation region are given in Table 1.

Table 1. *Distribution of Gram-Negative Anaerobic Bacteria by Isolation Region*

Isolation Region	*B. fragilis*	*Bacterioides spp.*	*Prevotella spp.*	*Fusobacterium spp.*	*Veillonella spp.*	*D. pneumosintes*	Total
Skin and soft tissue	0	1	0	0	1	0	2 (% 4,4)
infection	3	1	1	1	0	0	6 (% 13,3)
Intra-abdominal infection	1	0	2	0	0	0	3 (% 6,6)
Genital system infection	3	1	10	3	0	1	18 (% 40)
Head and neck infection	0	0	2	0	0	0	2 (% 4,4)
Respiratory tract infection	1	2	1	0	0	0	4 (% 8,8)
Bone and joint infections	3	1	6	0	0	0	10 (% 22,2)
Other infection							
Total	11	6	22	4	1	1	45 (% 100)

While the penicillin resistance of all isolated *Bacteroides* (17) species was determined to be 100%, 100% sensitivity to no antibiotic was observed. However, the antibiotics with the lowest antimicrobial resistance in *Bacteroides* species were determined to be imipenem (IP) and chloramphenicol (CL), with a rate of 5.9%. On the other hand, the antibiotics with the highest antimicrobial resistance were determined as penicillin (P) at 100%, clindamycin (CM) at 41.2% and amoxicillin-clavulanic acid (AMC) at 35.3%, respectively. In this study, some of the antibiotics with the lowest antimicrobial resistance of *Bacteroides* species were imipenem (IP) (5.9%), meropenem (MP) (17.7%), and metronidazole (MZ) (17.7%). However, although the antimicrobial sensitivity to these antibiotics was high, an increasing resistance was observed with the detection of bacterial colonies growing in the inhibition zone areas. While 100% antimicrobial resistance was

not observed against any applied antibiotic in *Prevotella* (22) species, 100% antimicrobial susceptibility was observed against imipenem (IP) and chloramphenicol (CL). On the other hand, the antibiotics with the highest antimicrobial resistance were determined as penicillin (P) at 45.5% and moxifloxacin (MX) at 27.3%. Although the antimicrobial susceptibility of some species to imipenem (IP) is 100%, an increasing resistance has been observed with the detection of bacterial colonies growing in the inhibition zone areas. In this study, only *Veillonella parvula* was isolated from *Veillonella* species, and resistance was observed against penicillin (P), imipenem (IP) and piperacillin-tazobactam (TPZ) antibiotics, while resistance was observed against amoxicillin-clavulanic acid (AMC), meropenem (MP), clindamycin (CM), no resistance was observed to the antibiotics moxifloxacin (MX), metronidazole (MZ), and chloramphenicol (CL). Similarly, while only metronidazole (MZ) resistance was observed in the isolated *Dialister pneumosintes* bacteria, penicillin (P), imipenem (IP), piperacillin-tazobactam (TPZ), amoxicillin-clavulanic acid (AMC), meropenem (MP), clindamycin (CM), no resistance to the antibiotics moxifloxacin (MX) and chloramphenicol (CL) was observed.

Table 2. *Distribution of Gram-Negative Anaerobic Bacteria*

Microorganisms	Number of Bacteria / %
Bacterioides spp.	17 (% 37,7)
Bacterioide fragilis	11
Bacteroides ovatus	3
Bacteroides thetaiotaomicron	2
Bacteroides vulgatus	1
Prevotella spp.	22 (%48,8)
Prevotella bivia	3
Prevotella disiens	2
Prevotella intermedia	1
Prevotella oris	2
Prevotella melaninogenica	1
Prevotella buccae	7
Prevotella denticola	3
Prevotella nigrescens	2
Prevotella baroniae	1
Fusobacterium nucleatum	4 (% 8,8)
Veillonella parvula	1 (% 2,2)
Dialister pneumosintes	1 (% 2,2)
Total	45 (% 100)

Conclusion

Since the identification of anaerobic bacteria and antimicrobial susceptibility tests are expensive and time-consuming, these procedures cannot be performed routinely in most laboratories. Various antibiotics used in the treatment of anaerobic infections over time and increasing bacterial resistance to them vary from region to region.[12–15] In our study, 48 anaerobic bacteria were isolated from 260 abscess samples, 9 anaerobic bacteria from 95 tissue/wound samples, 4 anaerobic bacteria from 6 blood samples, 1 anaerobic bacteria from 5 joint fluid samples, and 1 anaerobic bacteria from 2 urine samples. It has been stated in studies that the anatomical regions and clinical samples from which anaerobic bacteria are isolated may vary. Our study revealed that the sample processing procedure was compatible with previous studies. In our study, of the 45 Gram-negative anaerobic bacteria isolated from the infection sites, 11 (24.4%) were *Bacteroides fragilis* and 6 (13.3%) were other *Bacteroides* species, for a total of 17 bacterial species. According to EUCAST and CLSI MIC limit values, all of these bacteria were susceptible to penicillin (P), 7 (41.2%) to clindamycin (CM), 6 (35.3%) to amoxicillin-clavulanic acid (AMC), 4 (23%) to amoxicillin-clavulanic acid (AMC), 4 (% 236) to moxifloxacin (MX), 3 (17.7%) to meropeneme (MP) and metronidazole (MZ), 2 (11.7%) to piperacillin-tazobactam (TPZ), 1 (5%) to piperacillin-tazobactam (TPZ). While 1 (%5,9) were resistant to chloramphenicol (CL) and imipenem (IP), 1 (6%) was moderately sensitive to piperacillin-tazobactam (TPZ) and 3 (17.6%) was moderately sensitive to moxifloxacin (MX). However, 11 (64.7%) were susceptible to amoxicillin-clavulanic acid (AMC), 16 (94.1%) to imipenem (IP) and chloramphenicol (CL), and 14 (82.3%) to meropenem (MP), piperacillin-tazobactam (TPZ) and metronidazole (MZ), 10 (58.8%) to clindamycin (CM) and moxifloxacin (MX). In this study, the antibiotics to which *Bacteroides* species showed the highest resistance can be listed as penicillin (100%), clindamycin (41.2%), amoxicillin-clavulanic acid (35.3%) and moxifloxacin (23.6%). Neriman S. isolated 5 *Bacteroides* species in her thesis study in 2018. [16] According to EUCAST and CLSI MIC limit values, of these bacteria 4 (80%) were susceptible to penicillin (P), 3 (60 to chloramphenicol (CL), 1 (20%) to imipenem (IP) and metronidazole (MZ) was found to be resistant. In this study, while the results of penicillin and metronidazole were almost exactly compatible with the results of our study, they were also incompatible with the results of chloramphenicol and imipenem.

In our study, 22 (48.8%) of 45 Gram-negative anaerobic bacteria isolated from infection sites were *Prevotella* species. According to EUCAST and CLSI MIC limit values, 10 (45.5%) of these bacteria were susceptible to penicillin (P), 6 (27.3%) are susceptible to moxifloxacin (MX), and 5 (22.8%) wer susceptible to clindamycin (CM), 3 (13.7%) to metronidazole (MZ), 2 (9%) to

piperacillin-tazobactam (TPZ), 1 (4.5%) to amoxicillin-clavulanic acid (AMC) and meropenem (MP). Resistance to imipenem (IP) and chloromphenicol (CL) was not observed in any of these bacteria. In addition, 1 (4.5%) of these bacteria was moderately resistant to penicillin (P), and 2 (9%) were moderately resistant to piperacillin-tazobactam (TPZ). However, 11 (50%) were susceptible to penicillin (P), 21 (95.5%) to amoxicillin-clavulanic acid (AMC) and meropenem (MP), 18 (82%) to piperacillin-tazobactam (TPZ), 17 (77.2%) to clindamycin (CM), 16 (72.2%) to moxifloxacin (MX), and 19 (86.3%) to metronidazole (MZ). In our study, the antibiotics to which *Prevotella* species showed the highest resistance can be listed as penicillin (45.5%), clindamycin (22.8%), metronidazole (13.7%) and moxifloxacin (27.3%). Neriman S. isolated 3 *Prevotella* species in her thesis study in 2018. [16]. According to EUCAST and CLSI MIC limit values, 2 (66.6%) of these bacteria were resistant to penicillin (P) and chloramphenicol (CL), while 3 (100%) were sensitive to imipenem (IP) and metronidazole (MZ). All results in this study were incompatible with the results of our study. Bacalan et al. of the 69 anaerobic bacteria isolated in his study, 19 (27.5%) were *Prevotella* species.[17] According to CLSI MIC limit values, susceptibility results for only 23 anaerobic bacteria could be detected in this study. Among the *Prevotella* species for which results were obtained, penicillin (P) resistance was observed in only 2 species. These results were incompatible with the results of our study. In this study, according to CLSI and EUCAST MIC values, the antibiotics to which *Prevotella* species were resistant penicillin (77%), clindamycin (40%), amoxicillin-clavulanic acid (0%), imipenem (0%), meropenem (0%), metronidazole (%17. 7), piperacillin-tazobactam (3%) and moxifloxacin (30%). Again, according to the results of this study; In our study, the resistance rates to penicillin and clindamycin were low, while the resistance rates to amoxicillin-clavulanic acid, piperacillin-tazobactam, meropenem and metronidazole were found to be high, and they were also compatible with the resistance rates to imipenem and moxifloxacin. In our study, 4 (8.8%) of the 45 Gram-negative anaerobic bacteria isolated from infection sites were *Fusobacterium* species, 1 (2.2%) was *Veillonella parvula* and 1 (2.2%) was *Dialister pneumosintes*. Among these anaerobic bacteria, *Fusobacterium* species were sensitive to all antibiotics. However, *Veillonella* species were resistant to penicillin, piperacillin-tazobactam and imipenem, and *Dialister pneumosintes* was resistant only to metronidazole. Neriman S. in her thesis study in 2018, 2 *Fusobacterium*, 1 *Veillonella parvula* and 1 *Dialister pneumosintes* bacteria were isolated.[16] 1 Metronidazole resistance was observed in *Fusobacterium* and *Dialister pneumosintes*, penicillin resistance was observed in *Dialister pneumosintes* and *Veillonella parvula*, and chloromphenicol resistance was observed in *Veillonella parvula*, and these results were not compatible with the results of our study. Bacalan et al. Of the 69 anaerobic bacteria

isolated in his study, 14 (20.3%) were *Veillonella* and 3 (2.2%) were *Fusobacterium*. [17] According to CLSI MIC limit values, susceptibility results for only 23 anaerobic bacteria could be detected in this study. In this study, while 2 of the *Fusobacterium* were resistant to penicillin, no penicillin, clindamycin, imipenem and metronidazole resistance was observed in any of the *Veillonella*. These results were not compatible with the results of our study. In various studies conducted in different parts of the world, the majority of anaerobic bacteria isolated from clinical samples are *Bacteroides* species.[18–20] However, in different studies, the superiority of *Prevotella* species in number has been reported. [18,21] There are some changes in the resistance profile of anaerobes, particularly Bacteroides fragilis, to β-lactam antibiotics, as previously reported. [21–23] Although carbapenems are the most effective drugs against anaerobes, the emergence of resistance to them has been reported. Imipenem resistance due to metallo-β-lactam has been reported since 1986. [24] *Bacteroides* species are potentially resistant to most antibiotics, and this antibiotic resistance can also vary greatly between geographic regions and even healthcare facilities within the same area. Resistance rates can also vary greatly between different strains of the *Bacteroides fragilis* group.[25] In different studies, it has been reported that the variable resistance profiles of anaerobic bacteria against certain antibiotics may be related to the differences in the determined EUCAST and CLSI MIC limit values. [26]

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ORIGINAL RESEARCH

Determination of Antibacterial Efficacy of Lactoferrin Glycoprotein Obtained from Cow Colostrum

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Abstract

Objective: *It was aimed to investigate the antibacterial effect levels of Lactoferrin protein in cow colostrum. The aim of the study was to evaluate the effect of lactoferrin protein obtained from a natural product on the growth of Escherichia coli, Pseudomonas aerugenosa and Staphylococcus aureus.*

Method: *Lactoferrin at 4 different concentrations (100 mg/ml, 50 mg/ml, 25mg/ml and 12.5mg/ml) was impregnated on 6 mm discs prepared from filter paper and placed on the MHA plate and after incubation at 37˚C for 24 hours, inhibition was evaluated. The antibacterial effect levels of naturally obtained lactoferrin at different concentrations were compared.*

Results: *It was determined that the antibacterial activity of lactoferrin against Gram-negative bacteria Pseudomonas aeruginosa was higher than the other bacterial species in the study. It was determined that Lactoferrin at a concentration of 12.5mg/ml had the least inhibitory effect in the studied bacteria. It was concluded that E.coli and S. aureus were bacteria resistant to the antibacterial activity of lactoferrin.*

Conclusion: *It was observed that this effect was limited in E.coli bacteria. In the light of these results, it was concluded that Lactoferrin, an antibacterial agent, has different antibacterial effects depending on the bacterial species and dose.*

Keywords: Cow colostrum, lactoferrin, antibacterial effect and whey protein.

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Introduction

Lactoferrin (Lf) was identified in bovine milk in 1939 and isolated from both human and bovine milk in 1960[1]. Lactoferrin, a multifunctional iron-binding glycoprotein, plays an important role in immune regulation and defense mechanisms against bacteria, fungi and viruses[2]. Colostrum is the first milk produced by mammals after birth and has a high nutritional profile[3]. It is a complex biological fluid that provides the baby with all the necessary nutrients, immunological and developmental factors and is significantly different from mature milk[4]. Colostrum contains proteins, lipids, essential fatty acids and amino acids in greater concentration than mature milk. It also contains oligosaccharides, lactoferrin, lysozyme, lactoperoxidase, proline-rich polypeptides, insulin, growth hormone, cytokines and nucleosides, all of which act as natural anti-microbials[5,6]. The fact that lactoferrin has a high iron binding ability and is the only protein in the transferrin family that can show this ability in a wide pH range, especially at very low pH values, is highly resistant to proteolysis, has a net positive charge and is found in many tissues[7]. Colostrum has the feature of preventing infections and diseases in humans and provides passive immunity to human and bovine newborns[8]. Bovine colostrum is very often used as a dietary supplement. Clinical studies have revealed that colostrum is taken by humans as supplements; exhibits antioxidant, immunomodulatory and anti-inflammatory activity[9]. Lf is 80-kD, and the protein consisting of 692 amino acids consists of a single polypeptide chain with 2 symmetrical folds to which it is glycosylated[10]. In most cases, enzymes activate the milk's natural antibacterial system and kill target microbes. The antimicrobial systems in natural milk are distinguished by their simultaneous attack on the oxidative and lytic mechanisms of microorganisms[11]. The second mechanism of lactoferrin and lactoferrin-derived peptides is in direct interaction with microorganisms through positively charged amino acids that interact with anionic molecules on certain bacterial, viral, fungal and parasitic surfaces, causing cell lysis[12]. Lactoferrin has high levels of amylase, DNase, RNase and ATPase activity. Therefore, Lf can damage the nucleic acids of bacteria through hydrolysis and inhibit the metabolism of the organism[13]. The antibacterial activity of lactoferrin varies depending on its concentration, interaction with other minerals in the environment, and the degree of saturation with iron. The antimicrobial activity of lactoferrin decreases when other macromolecules are bound and saturated with iron, but increases when it is found together with other antimicrobial proteins (lysozyme)[14]. It was determined that the main reason for the increase in antimicrobial activity was lactoferricin, a bioactive peptide released during enzymatic degradation. Lactoferricin is the 25 amino acid portion containing amino acids 18-42 at the N-terminus of cow's milk lactoferrin. The lactoferricin molecule folds into two globular units and each can bind one iron ion (Fe+3)[15].

Because of the bioactive components of colostrum, it may be useful in this study, reinforcing the notion that Lactoferrin is a significant barrier in the mucosal wall that is effective against bacterial infection attacks in terms of its capacity to exert a potent antibacterial activity through binding to host cells or viral particles. It can be applied as a new strategy in the treatment of pathogenic bacterial infections.

Materials and Method

This study was carried out in Dicle University Hospital Central Laboratory. In our study, after separating cow colostrum into whey protein fractions, lactoferrin protein was obtained as concentrated by using ultrafiltration membrane filter at 30kD. Total protein concentrations were measured according to the method of bicinchoninic acid colorimetric assay (BCA, Bio-Rad, USA). After lyophilization, the protein was ground into powder. Protein concentrations were measured according to the method of bicinchoninic acid colorimetric assay (BCA, Bio-Rad, USA). 10 µl of sample was taken and 40 µl of PBS was added and diluted 1/5. It was homogenized and 7 standards were added to 96 well wells and then two replicates of 20 µl samples were added respectively. After the auxiliary solutions were prepared in a sterile falcon tube, 160 µl was added to all wells, including the standard wells. The plate was closed and incubated in an oven at 37 ^{0}C for 30 minutes. Then, absorbance was measured at a wavelength of 562 nm in the Elisa Reader Thermo Scientific device.

For antibacterial analysis; Escherichia coli ATCC 25922 and Pseudomonas aerugenosa ATCC 27853 were used as Gram negative organisms, Staphylococcus aureus ATCC 29213 as Gram positive organisms. Tested bacterial strains were proliferated by incubating at 350°C in tubes containing Tryptic Soy Broth (TSB). 0.5 McFarland standard bacterial suspension was prepared from the bacterial culture obtained after 24 hours of incubation. Bacteria taken from the bacterial suspension using sterile cotton swabs were spread on the Mueller-Hinton Agar (MHA) surface and inoculated. Whatman No. Lactoferrin at 4 different concentrations (100 mg/ml, 50 mg/ml, 25mg/ml and 12.5mg/ml) was impregnated on 6 mm discs prepared from 1 filter paper and placed on the MHA plate. Inhibition was evaluated after the Petri dishes were incubated at 37˚C for 24 hours. A 1 mg/ml Ceftriaxone disc was used as a positive control. The antibacterial effect levels of naturally obtained lactoferrin at different concentrations were compared.

Statistical analyses

All analyzes were performed in duplicate and the data obtained as a result of the analyzes were subjected to analysis of variance (ANOVA) using the SPSS 22.0.0 package program. To compare the effects of statistically different concentrations, Duncan's multiple range test was analyzed at a significance level of $P < 0.05$.

Results

The antibacterial activity of lactoferrin was conducted on Gram-positive and Gram-negative bacterial strains. Total protein concentrations were measured according to the method of bicinchoninic acid colorimetric assay (BCA, Bio-Rad, USA). In these measurements, mean and standard deviation values at 100 mg/ml concentration were: 6894.5±21.99; 2513±36.77 at 50 mg/ml concentration; The values of 1154±35.35 at 25 mg/ml concentration and 923.5±0.70 at 12.5mg/ml concentration were determined statistically (Figures 1 and 2). Different concentrations of lactoferrin were prepared as the samples were 100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5mg/ml. 1 mg/ml Ceftriaxone was used as a positive control. It was determined that the antibacterial activity of lactoferrin against Gram-negative bacteria Pseudomonas aeruginosa was higher than the other bacterial species in the study. It was observed that its antibacterial activity was limited in E.coli bacteria. It was determined that Lactoferrin at a concentration of 12.5mg/ml had the least inhibitory effect in the studied bacteria. It was concluded that E.coli and S. aureus were bacteria resistant to the antibacterial activity of lactoferrin. (Pictures 1,2 and 3). It was determined that lactoferrin applied in Pseudomonas aeruginosa bacterial species inhibited bacterial proliferation at high concentration. Matijasic et al., (2020) investigated the antimicrobial effects of lactoferrin isolated from whey and produced in the pilot plant by disc diffusion method. The bacteria examined were Escherichia coli, Staphylococcus aureus, Listeria monocytogenes, Salmonella enterica, Clostridium difficile, Klebsiella oxytoca and Clostridium perfringens. As a result of the study, partial or complete inhibitory properties were observed on all bacteria except lactic acid bacteria and Clostridium bacteria[16]. In this study, lactoferrin isolated from whey showed an inhibitory effect against Pseudomonas aeruginosa bacteria. The antibacterial activity of lactoferrin varies depending on the protein concentration and the degree of saturation with iron. The activity level of these antimicrobial molecules decreases when they are saturated with iron, and increases when they are found together with other antimicrobial proteins (lysozyme)[17]. Lactoferrin has serine protease activity and can degrade the arginine-rich portion of proteins. It can show antibacterial activity by degrading lactoferrin with many serine protease activities[18]. Lactoferrin; It also causes inhibition by triggering the fragmentation of intracellular invasive plasmid antigens such as Escherichia coli, Listeria monocytogenes and Shigella flexneri[19]. In fact, it has been demonstrated that the candidacidal activity of lactoferrampin is higher than that of lactoferrin and that it has antibacterial activity against many bacteria such as Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa[20]. The inhibition effect of lactoferrin obtained from the cow colostrum fraction against Escherichia coli and Pseudomonas aeruginosa bacteria is in parallel with the above study.

Table 1. *Data Obtained by BCA Method of Concentrated Lactoferrin from Cow Whey Protein*

S_1	S_2	S_3	S_4	S_5	S_6	S_7	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml
2000	1500	1000	750	500	250	125	6739	2487	1129	924.8
2000	1500	1000	750	500	250	125	7050	2539	1179	923

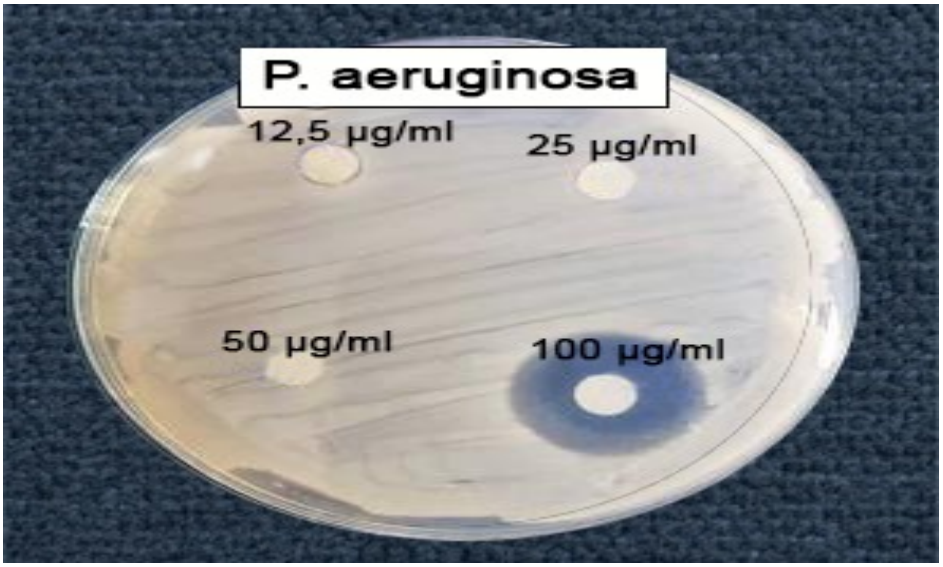
S_1, S_2, S_3, S_4, S_5, S_6, S_7 : BCA Yönteminde Kullanılan Standartlar

Table 2. *Statistical Analysis of Lactoferrin BCA Total Protein*

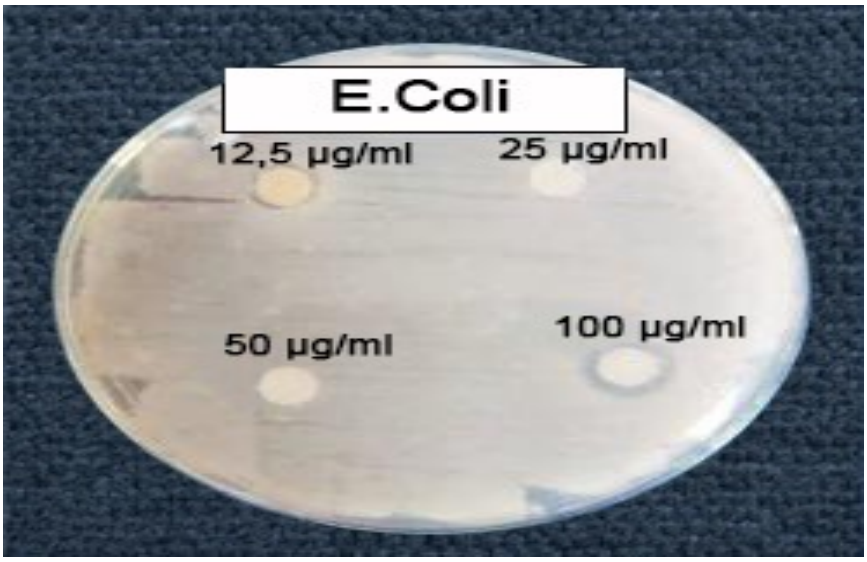
S_1 Mean±Sd	S_2	S_3	S_4	S_5	S_6	S_7
2000[a]	1500[a]	1000[a]	750[a]	500[a]	250[a]	125[a]
P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05

100mg/ml	50mg/ml	25mg/ml	12.5mg/ml
6894.5±21.99[a]	2513±36.77[a]	1154±35.35[a]	923.5±0.7[b]
P<0.05	P<0.05	P<0.05	P<0.05

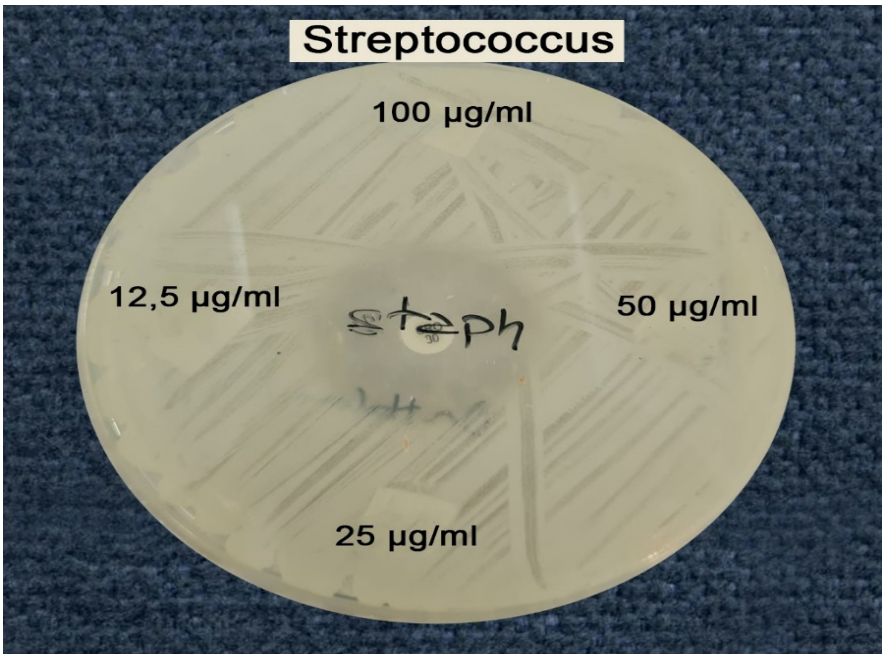
[a,b]Absorbanslar arasında anlamlılık düzeyi faklı olanlar aynı harflerle ifade edilmiştir.



Picture 1. *Effect of Lactoferrin on P.aeruginosa Bacteria at Different Doses*



Picture 2. *Effect of Lactoferrin on E.coli Bacteria at Different Doses*



Picture 3. *Staphylococcus aureus Effect level of Lactoferrin at different doses*

Conclusion:

Lactoferrin glycoprotein obtained from bovine colostrum has a significant and dose-dependent antibacterial effect on the growth of P. aeruginosa. In this study, we think that the inhibitory effect against Pseudomonas aeruginosa bacteria may be due to the serine protease activity of this protein. In our study, we determined that the inhibition effect of Lf on E. coli bacteria was limited. Lactoferrampin, determined from the N1 domain of cow's milk lactoferrin, was also found to have antimicrobial activity. S. aureus bacteria were found to be resistant to all doses of lactoferrin

protein. In the light of these results, it was concluded that Lactoferrin, an antibacterial agent, has different antibacterial effects depending on the bacterial species and dose.

Declaration of Conflicting Interests: The authors declare that they have no conflict of interest.

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ORIGINAL RESEARCH

Long Non-Coding RNA H19 Expression in Leukemia Patients

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Abstract

Objective: *BCR-ABL fusion gene occurs with the formation of translocations in the t(9;22) region of the Philadelphia (Ph) chromosome, which is used as a diagnostic biomarker in Chronic Myeloid Leukemia (CML). These abnormal genetic changes, which cover 15% of leukemias, reach dimensions that threaten human life. Recent studies have determined that thousands of genes expressed in differentiation and development processes contain non-protein-coding RNA with a regulatory role. Of these, the first discovered long noncoding RNA (LncRNA) H19 has been associated with its biological role, cell proliferation, apoptosis and metastasis Therefore, studies have been conducted considering that LncRNA H19 can be used as a biological marker in CML patients.*

Method: *For this study, blood from 72 CML patients over 18 years of age and 64 healthy individuals were used. After RNA isolation of each of these bloods and cDNAs were obtained, the expression levels of the LncRNA H19 gene were analyzed by Real Time PCR method.*

Results: *As a result of the expression study, it was found that LncRNA H19 gene expression increased 4.37 times and was upregulated in Bcr-Abl positive patients (p=0.414683).*

Conclusion: *In this study conducted in Diyarbakır region, we think that LncRNA H19, which is up-regulated in terms of CML profiles, can be used as a biological marker for new treatment applications.*

Keywords: *CML, LncRNA, H19, RT-PCR, BCR-ABL*

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Introduction

CML is a malignant disease that occurs with an uncontrolled increase in the number of leukocytes produced in the blood and bone marrow and generally affects the life of people over 40 years of age negatively[1]**.** BCR-ABL fusion gene occurs with the formation of translocations in the t(9;22) region of the Philadelphia (Ph) chromosome, which is seen in 95% of CML cases[2]. Diagnosis is made by detecting the BCR-ABL fusion gene from blood samples taken from CML cases by RT-PCR method[3]. As a result of the analyzes obtained from the genome studies, it has been determined that thousands of genes contain non-protein-coding RNA with a regulatory role[4]. In addition, LncRNA H19 contributes to the formation and progression of CML in tumor formation of the BCR-ABL gene, which is an oncogene. LncRNA H19 has been identified as functional involvement in the progression of BCR-ABL mediated leukemia[5]. Lnc RNA H19 found in humans has been identified as a maternally expressed imprinted gene with a length of 2.7 kb (NR_002196, 2322 bp RNA) and four small intron regions[6]. Excessive increase in the expression of LncRNA H19 in various cancer types causes uncontrolled proliferation of cells and spread of cancer. Therefore, LncRNA H19 has been shown to be effective in the uncontrolled proliferation of cells, programmed cell death, and the spread of cancer throughout the body[7].

The specific mechanism of lncRNA H19 in CML remains to be elucidated. We think that the results of our study for the expression of LncRNA H19 in CML patients will contribute to further studies.

Materials and Methods

Our study, which was approved by Dicle University, started in August 2022 according to the Declaration of Helsinki and was concluded in February 2023. The study included 72 patients who applied to the Hematology outpatient clinic of Dicle University Medical Faculty Hospitals with the diagnosis of CML or were followed up with a new diagnosis, and 64 healthy individuals without any health problems as the control group were included in the study. Two tubes of 10 ml of blood were taken from the individuals included in the study. Whole blood White Blood Cell (WBC) results of the patients were recorded from the hospital automation system for leukocyte isolation from the blood sample. According to the RNA isolation kit protocol, the required amount of blood samples were taken by looking at the WBC counts, and leukocyte and RNA isolation were performed. The concentration values of the RNAs we obtained were measured in a spectrophotometer.

The cDNA was obtained according to the Ipsogen RT Kit (Qiagen GmbH, 679923) protocol used for cDNA extraction from the RNA isolated samples. After this step, BCR-ABL positive

patients were identified using the Ipsogen BCR-ABL1 Mbcr IS-MMR Kit with the RT-PCR method. The cDNAs of the determined samples were diluted 1/20 and RT-PCR was performed with the LncRNA H19 primer and B2M (Beta-2-microglobulin) as the reference gene, since it showed high stability. Information about the B2M reference gene and H19 primers used in the study were obtained from the National Library of Medicine and are indicated in Table 1[8].

Table 1. *Information on the B2M reference gene and H19 primers*

Position	RefSeq Number	GeneID	MIM	Symbol	Description
1	NR_002196	283120	103280	H19	LncRNA
2	NM_004048	567	109700	B2M	Beta-2-mikroglobulin

RT-PCR Analysis

For our study, SYBR Green qPCR Mastermix, Human LncRNA H19 qPCR Assay and B2M (LPH28472A) as Reference Gene (House Keeping) were used. During the mixing phase for RT-PCR, 12.5 µl of Sybr Green, 1 µl of H19 primer and 10.5 µl of ultrapure water were prepared per sample. 1 µl of the relevant sample cDNA was added to the prepared total mixture of 24 µl and it was determined as 25 µl. For the RT-qPCR procedure, the PCR protocol was applied as the first heating at 95 °C for 10 minutes and 40 cycles at 95 °C for 15 seconds and 60 °C for 30 seconds. Ct values were determined by evaluating all samples used in the study. GeneGlobe Data Analysis was used for the analysis of the Ct values found. The B2M reference gene was selected to normalize Ct values. Negative Control (NC: Rnase Free Water) was used to see if a contamination had occurred in the samples. Data from the RT-PCR device are shown in Figure 1 below.

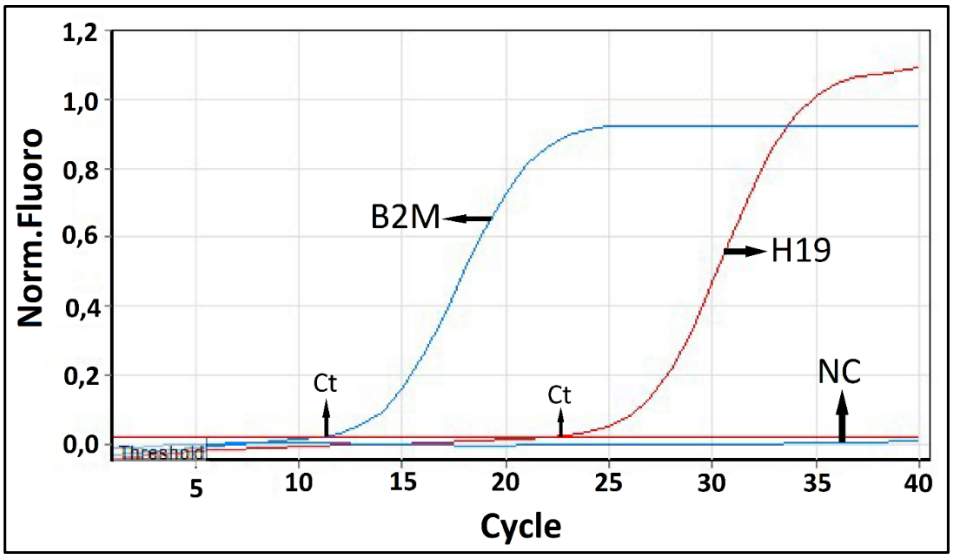

Figure 1. *RT-PCR data*

Statistical Analysis

Fold Change

According to the statistical analysis of our study; Our fold change value, which we found as a result of the comparison of the sick and healthy samples, is 4.37. This is considered to be greater than 1.00, which is accepted as the cut-off value, and significantly increased gene expression according to the p-value < 0.05, and these values are expressed in Table 2. The p-value is based on the deviation between the observed H19 value and the reference B2M, considering the probability data of our study. Statistical analysis was performed online at the GeneGlobe Data Analysis Center[9].

Table2. *Analysis data for H19 and B2M*

Position	Gene Symbol	Fold Change	p-Value
1	H19	4,37	0,414683
2	B2M	1	nan

In our study, the distribution of the change in the expression of LncRNA H19 in CML patients was compared with the data normalized with the B2M gene in RT-PCR analysis. In the figure below, it is seen that B2M, which is used as a reference gene, has not changed since it is located in the central diagonal. The other two diagonals show the threshold of the floor change. Since the point indicated by H19 is outside these thresholds, it was observed that it was upregulated (Figure 2).

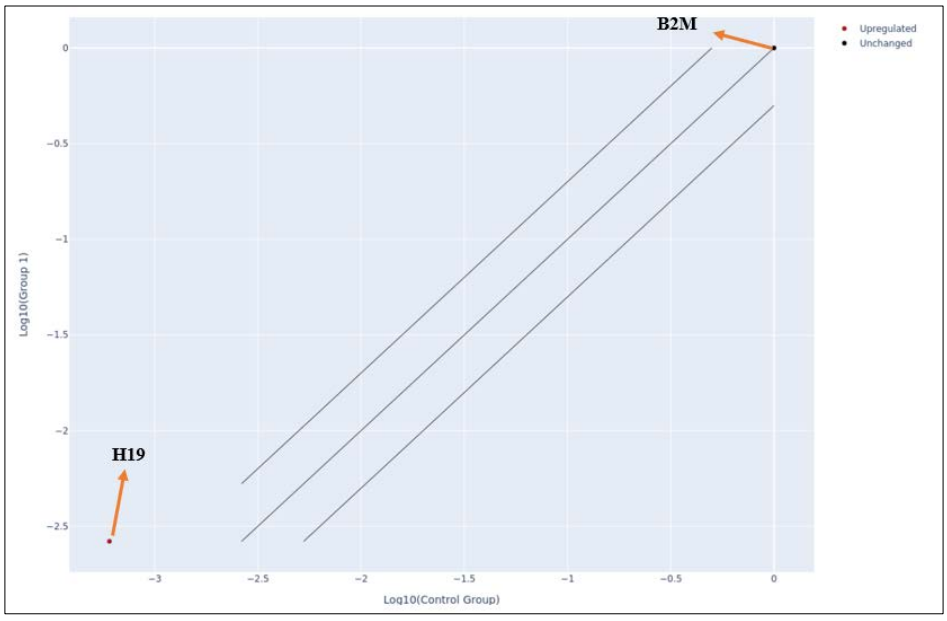

Figure2. *Scatterplot of H19 and B2M*

Results

In the light of previous studies on the H19 gene, the expression of the H19 gene was investigated in 72 CML patients over 18 years of age and 64 healthy individuals who applied to the outpatient clinic for the diagnosis of CML. For this reason, it was determined that the expression of LncRNA H19 was upregulated in individuals with positive Bcr-Abl results.

Discussion

The diagnosis of CML is made by detecting the Bcr-Abl oncogene from the samples taken by applying the RT-qPCR method[3].

In studies on the human genome, it has been determined that thousands of genes contain non-protein-coding RNA with a regulatory role as a result of whole genome and functional analysis[4]**.** Many non-coding miRnas and LncRNAs were found by Klattenhoff et al. It has been stated that lncRNAs are expressed in differentiation and development processes and have important roles in controlling some cellular processes[10].

Thanks to recent technological applications, it has been reported that many transcription factors, miRNAs, RNA-binding proteins and regulators that promote or inhibit tumor growth after transcription, such as lncRNAs, are very important in studies comparing cancer cells and normal cells related to the increase in cancer[11,12,13].

We think that the identification of lncRNA H19, which contributes to tumor formation and is involved in cellular processes during the cancerization process, will contribute to therapeutic targets used in cancer treatment. These non-protein-forming RNAs can be the starting point for the treatment process for the development and prevention of cancer. It can be said that lncRNAs provide answers to questions about the molecular pathology and genetics of cancer formation.

Conclusion

In summary, our data on maternally expressed LncRNA H19 showed upregulation in positive BCR-ABL patients diagnosed with CML. Therefore, we think that it will be beneficial to find out the effects and duties of LncRNAs, including H19, in various types of cancer in humans, and to further elucidate their roles. Therefore, many new findings are needed to determine the expression and targets of other LncRNAs.

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ORIGINAL RESEARCH

The Effect of Arm Length and Hand Quickness on Shot Accuracy Rates in Basketball Players

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Abstract

Objective: *The effects of various physical and motor skills on the performance of basketball players are well known. Scoring through shooting is one of the most commonly used techniques in basketball. This study analysed the connection between arm length and hand quickness among basketball players and their shooting accuracy.*

Method: *The study was carried out on 17 male and 16 female athletes, aged 18-25 years, who played basketball in university teams in 2021-2022 and had at least 5 years of eligibility. The athletes' height, body weight and full length of each athlete's arm were measured. The AAHPERD Basketball Speed Spot Shooting Test was used to measure the shooting accuracy of the athletes. For the athletes' hand quickness, Hand Quickness Test (Touching Discs) was used.*

Results: *Results indicated a moderate positive correlation between hand quickness score and shot accuracy score among male athletes, but this relationship was not statistically significant. However, a moderate positive and statistically significant correlation was found between hand quickness score and shot accuracy score among female athletes. Among all athletes, there was a positive and statistically significant correlation between arm length, hand quickness speed, and shot accuracy score.*

Conclusion: *The study's findings indicated that shooting accuracy was also impacted by arm length, with particular emphasis on hand quickness of the athletes.*

Keywords: *Basketball, Arm length, Hand quickness, Shooting accuracy*

Introduction

Morphological characteristics not only play a key role in shaping human movement, but also have a significant impact on athletic performance. Commonly studied topics within the field of morphology include dimensions such as length and width, environmental factors and body composition [1].

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Sport is a comprehensive term encompassing all physical or mental activities, undertaken either individually or as a team according to predetermined guidelines, predominantly reliant on competitive play and personal pleasure or striving for excellence [2].

The demands of everyday life necessitate motor skills, including strength, endurance, speed, flexibility, coordination, and proprioception, which involve the pushing, pulling, rotating, and level changes required for human movement [3].

Basic motoric characteristics determine an individual's physical strength and ability, as well as their degree of motoric sports power of a complex nature. These characteristics form the foundation and primary condition of every motoric sports movement performed during training. The order of importance for basic motoric characteristics is strength, endurance, speed, mobility, and skill, with the first three being main characteristics and the latter two being complementary [4].

Basketball requires physical, technical and mental qualities, as well as some branch-specific skills, including tactical knowledge. Athletes' high physical competence facilitates efficient offensive and defensive game strategies. These talents are essential in basketball since triumph is highly coveted [5]. Basic skills such as shooting, dribbling, and passing are crucial factors affecting success in basketball. The technical aspect of shooting, which is a movement towards scoring points, is often included in research. The shooting movement is considered to have commenced from the moment the ball leaves the athlete's hand, and the shooting mechanics must adhere to specific rules from the first movement. The accuracy of a shot relies on various factors, including the shot's height, the velocity at which the ball departs the athlete's hand, and the shot angle [6].

Testing arm movement speeds and skills in basketball with a focus on shooting accuracy is believed to enhance efficiency in the sport. This study aims to evaluate the arm movement speed levels of male and female basketball players in the basketball teams at Diyarbakır Dicle University and examine potential correlations with age, gender, height, weight, arm length, and shooting score.

Materıals and Methods

17 male and 16 female basketball players who had been playing basketball for at least 5 years voluntarily participated in the study. The research implemented the experimental design method, one of the quantitative research techniques.

The study's population comprised male and female basketball players from university teams during the 2021-2022 season. The research group was determined through a typical case sampling method utilizing purposeful (judgemental) sampling, a type of non-random sampling. Specifically,

the research group consisted of male and female athletes aged 18-25 playing for Dicle University's basketball teams.

Before administering the tests to the athletes, the relevant instructions were read and the measuring equipment was introduced. The athletes underwent a single trial for the measurements, and the data obtained from these measurements were recorded on the measurement forms. The measurements were performed at the indoor sports hall of Prof. Dr. Şeref İNALÖZ in Dicle University.

The athletes' height and body weight were measured using a SECA height and weight meter, with an accuracy of 0.1. The full length of each athlete's arm was measured using a Martin Type Anthropometer, which requires two people to operate. The measurers stood on the left side of each subject, with one holding the subject's arm and hand slightly forward and to the side, thereby assisting the subject in attaining the full length of their left arm. The individual conducting the measurement positions the anthropometer's horizontal arm on the subject's acromiale point. The other person helps the arm reach its full length by lightly touching the anthropometer's other horizontal arm to the tip of the subject's longest finger (dactylion point). It is important to ensure that the axes of the arm and the anthropometer are parallel to each other.

The AAHPERD Basketball Speed Spot Shooting Test was used to measure the shooting accuracy of the athletes. The test comprises specific exercises pertaining to dribbling, passing, shooting, and defensive movements. The coefficient of validity for all sections of the test was reported to be 0.65-0.95. Its reliability was tested through a retest study, which indicated a reliability coefficient of 0.84-0.97 [7]. The shooting abilities of youthful basketball players were measured in this research by utilising the quick point shooting section of the test battery. According to this test protocol;

Five shooting points are positioned at equal intervals, 4.57 metres from the centre of the half-court hoop on a basketball court according to international standards. The points are measured from the projection of the hoop's centre and marked with 60 cm clear tape. The athlete begins the shooting test from the starting point. The athlete takes a shot at the hoop from the first point, retrieves the ball, moves to the next shooting location, and continues the pattern until they have shot from each of the five locations. It is required that one foot of the athlete stays behind the marking line while shooting. The athlete is permitted to attempt a lay-up upon retrieving a missed shot but is prohibited from consecutively attempting two lay-ups. During the test, the athlete shoots or completes a lay-up from five different points on the court until the "stop" warning is given. Once 60 seconds have passed, the shooting test concludes. Technical abbreviations will be explained when

initially used. Two points are granted for each successful shot or lay-up, while a missed shot that hits the rim and returns will receive one point. If the lay-up is successfully made after the ball returns from the hoop, two points will be awarded. If two consecutive lay-ups are made correctly, no points will be assigned for the second one. Up to four lay-ups may be attempted within a 60-second time frame, and no additional points will be granted beyond this. Rule infractions, like dribbling, ball handling, and crossing the shooting line, will not earn any points [7, 8].

Hand Quickness Test (Touching Discs); Two plastic discs with a diameter of 20 cm are positioned on the table, with the centre points of the two discs being 80 cm apart (with the edges of the discs being 60 cm apart). A rectangular plate is placed at an even distance from the two discs, measuring 10 x 20 cm. The dominant hand is crossed over the other hand and placed on the disc in the opposing direction to it. The test requires the athlete to move their preferred hand placed on the disc over the other hand and touch the discs as quickly as possible. The test is performed twice, and the best performance is recorded as the final result. Attention is paid to the disc on which the athlete places the preferred hand at the beginning of the test, and the touches made on this disc are counted. The test adheres to objective measures and ensures a logical progression of information. Attention is paid to the disc on which the athlete places the preferred hand at the beginning of the test, and the touches made on this disc are counted. Assuming the test commences when the subject touches disc A, the stopwatch is terminated when the subject touches disc A 25 times. This means that the total number of contacts on discs A and B is 50. The score is a measurement of the time needed for a total of 50 touches in units of 1/10. For instance, finishing the test in 10.3 seconds for 25 cycles gives a score of 103 points [9].

Our research project was reviewed by the Ethics Committee for Social and Human Sciences at Dicle University, in compliance with the Higher Education Institutions' guidelines for Scientific Study and Publication Ethics. The study was found to adhere to scientific ethical standards and received approval on 08.03.2022 with reference number 244973.

Statistical Analysis: The data was statistically analysed using SPSS 22.0. Normality tests were conducted by examining skewness and kurtosis values. For non-normally distributed data, Mann Whitney U test was applied. In addition, Pearson correlation analysis was used to test the relationship between variables [8].

Results

Descriptive statistical analysis was utilised to determine the frequencies and percentages of athletes according to gender. The outcomes obtained are presented in Table 1.

Table 1. *Descriptive Statistics of Athletes by Gender*

Variables	n	%
Male	17	51,5
Female	16	48,5
Total	33	100

n: number, **%**:percentage

The study comprised 33 athletes, 17 of whom were males (51.5%) and 16 of whom were females (48.5%), as our analysis revealed.

Descriptive statistical analysis was conducted to calculate the mean and standard deviation of the age, height, weight, years of experience in sports, arm length, shot accuracy score, and hand quickness score of the athletes. The obtained values are presented in Table 2.

Table 2. *Descriptive Statistics for Age, Height, Weight, Sport Year, Arm Length, Shooting Accuracy Score, and Hand Quickness Score among Athletes*

Variables	Male	Female	Total
Age (years)	21,00 ± 1,00	21,31 ± 1,07	21,15 ± 1,03
Height (cm)	186,00 ± 8,35	170,62 ± 5,71	178,54 ± 10,53
Weight (kg)	76,47 ± 9,95	58,25 ± 5,73	67,63 ± 12,26
Sport Year (years)	10,17 ± 1,55	10,25 ± 1,61	10,21 ± 1,55
Arm Length (cm)	81,11 ± 4,13	72,87 ± 4,11	77,12 ± 5,82
Hand Quickness Score	95,94 ± 8,23	116,56 ± 12,60	105,93 ± 14,76
Shot Accuracy Score	17,35 ± 1,41	13,81 ± 3,14	15,63 ± 2,97

The mean values for age, height, weight, years of sports experience, arm length, shot accuracy scores, and hand quickness scores were compared between male and female athletes using an Independent Sample T Test. The study results are presented in Table 3 ($p<0.05$).

Table 3. *Comparison of Average Age, Height, Weight, Sport Year, Arm Length, Shooting Accuracy Score, and Hand Quickness Score Among Athletes by Gender*

Variables	Male	Female	p
Age (years)	21,00 ± 1,00	21,31 ± 1,07	,394
Height (cm)	186,00 ± 8,35	170,62 ± 5,71	,000**
Weight (kg)	76,47 ± 9,95	58,25 ± 5,73	,000**
Sport Year (years)	10,17 ± 1,55	10,25 ± 1,61	,895
Arm Length (cm)	81,11 ± 4,13	72,87 ± 4,11	,000**
Hand Quickness Score	95,94 ± 8,23	116,56 ± 12,60	,000**
Shot Accuracy Score	17,35 ± 1,41	13,81 ± 3,14	,000**

p<0,05*****, p<0,01******

Table 3 indicates a statistically significant differentiation in height, weight, arm length, hand quickness score, and shooting accuracy score ($p<0.05$, $p<0.01$). However, no statistically significant distinctions were observed between the groups in terms of age and sport year parameters ($p>0.05$).

Pearson's correlation analysis was utilised to ascertain the correlation between the athletes' demographic data, arm length, hand quickness score and shooting score. The results are presented in Table 4.

Table 4. *Exploring the Correlation between Athletes' Demographic Information, Arm Length, Hand Quickness Score, and Shooting Score*

Variables		**Gender**	**Age**	**Height**	**Weight**	**(SY)**	**(AL)**	**(HQS)**	**(SAS)**
Gender	Pearson r	1	,153	-,740**	-,754**	,024	-,718**	-,709**	-,604**
	p		,394	,000	,000	,895	,000	,000	,000
Age	Pearson r		1	-,045	-,109	,814**	-,138	-,267	-,195
	p			,803	,547	,000	,444	,134	,278
Height	Pearson r			1	,807**	,120	,955**	,616**	,402*
	p				,000	,505	,000	,000	,020
Weight	Pearson r				1	,034	,830**	,629**	,665**
	p					,853	,000	,000	,000
Sport Year (SY)	Pearson r					1	-,020	,050	-,010
	p						,911	,783	,957
Arm Length (AL)	Pearson r						1	,532**	,395*
	p							,001	,023
Hand Quickness Score (HQS)	Pearson r							1	,743**
	p								,000
Shot Accuracy Score (SAS)	Pearson r								1
	p								

******. Correlation is significant at p<0.01 level.
*****. Correlation is significant at p<0,05 level.

There was a statistically significant, negative correlation between gender and various scores including height, weight, arm length, hand quickness, and shooting accuracy (p<0.05, p<0.01). However, other parameters did not show a statistically significant difference between gender (p>0.05).

There was a significantly strong positive correlation between age and years of participating in sports (p<0.01). However, no statistically significant difference was observed between age and other assessed parameters (p>0.05).

There was a positive correlation between height and weight, arm length, hand quickness score and shooting accuracy score, which was statistically significant (p<0.05, p<0.01). However, there was no statistically significant difference between height and other parameters (p>0.05).

There was a significant, positive correlation between weight and arm length, hand quickness score and shooting accuracy score ($p<0.01$).

However, the difference between sport year and other parameters was not statistically significant ($p>0.05$).

There was a statistically significant and positive correlation observed between arm length, hand quickness score, and shot accuracy score ($p<0.05$, $p<0.01$). However, no significant correlation was found between arm length and other parameters ($p>0.05$).

A statistically significant and positive difference was found between hand quickness score and shot accuracy score, with a strong effect size ($p<0.01$).

Pearson correlation analysis was used to determine the relationship between demographic information and arm length, hand speed score and shooting score of male athletes. The results are presented in Table 5.

Table 5. *The Correlation Between Demographic Information of Male Athletes and Arm Length, Hand Quickness Score, and Shooting Score.*

Variables		**Age**	**Height**	**Weight**	**(SY)**	**(AL)**	**(HQS)**	**(SAS)**
Age	Pearson r	1	,195	,169	,806**	,076	,342	,443
	p		,454	,516	,000	,773	,180	,075
Height	Pearson r		1	,691**	,367	,890**	,618**	,329
	p			,002	,148	,000	,008	,198
Weight	Pearson r			1	,286	,807**	,371	,579*
	p				,266	,000	,143	,015
Sport Year (SY)	Pearson r				1	,143	,709**	,569*
	p					,585	,001	,017
Arm Length (AL)	Pearson r					1	,376	,314
	p						,137	,220
Hand Quickness Score (HQS)	Pearson r						1	,407
	p							,105
Shot Accuracy Score (SAS)	Pearson r							1
	p							

******. Correlation is significant at $p<0.01$ level.
*. Correlation is significant at $p<0,05$ level.

There was a significant and positive correlation between age and duration of sport participation among male athletes ($p<0.01$). However, the relationship between age and other variables did not demonstrate statistical significance ($p>0.05$).

A statistically significant and positive correlation was found between height and weight, arm length and hand speed score in male athletes ($p<0.01$). However, there was no statistically significant distinction between height and other parameters ($p>0.05$).

There was a significant and positive difference found between male athletes' weight, arm length, and their shooting accuracy score ($p<0.05$). However, there was no statistical significance found between weight and other parameters ($p>0.05$).

There was a statistically significant positive correlation between the hand quickness score and the shooting accuracy score of male athletes with years of sport involvement ($p<0.05$).

In male athletes, a moderate positive correlation was also observed between the hand quickness score and the shot accuracy score. However, this correlation was not statistically significant ($p<0.05$).

Pearson correlation analysis was employed to ascertain the correlation between the demographic data and arm length, hand quickness score, and shooting score of female athletes. A summary of the results is presented in Table 6.

Table 6. *The Correlation Between Demographic Data of Female Athletes and Arm Length, Hand Quickness Score and Shooting Score*

Variables		**Age**	**Height**	**Weight**	**(SY)**	**(AL)**	**(HQS)**	**(SAS)**
Age	Pearson r	1	-,023	-,262	,834**	-,156	-,600*	-,394
	p		,933	,328	,000	,564	,014	,131
Height	Pearson r		1	,225	-,025	,968**	-,211	-,397
	p			,402	,926	,000	,434	,128
Weight	Pearson r			1	-,281	,369	,055	,428
	p				,291	,160	,841	,098
Sport Year (SY)	Pearson r				1	-,156	-,314	-,253
	p					,564	,236	,344
Arm Length (AL)	Pearson r					1	-,180	-,260
	p						,504	,332
Hand Quickness Score (HQS)	Pearson r						1	,618*
	p							,011
Shot Accuracy Score (SAS)	Pearson r							1
	p							

******. Correlation is significant at $p<0.01$ level.
*****. Correlation is significant at $p<0,05$ level.

Conversely, a significant negative correlation was noted between hand quickness score and age ($p<0.05$).

Significant positive correlations were observed between the age and years of sport, as well as between the height and arm length of female athletes ($p<0.05$).

The disparities between weight and other factors in female athletes failed to attain statistical significance ($p>0.05$).

Similarly, the variances between years of sport and other parameters in female athletes did not display any statistical significance ($p>0.05$).

There were no statistically significant differences between arm length and other parameters in female athletes ($p>0.05$).

However, there was a moderate positive and statistically significant difference discovered between female athletes' hand quickness scores and shot accuracy scores ($p<0.05$).

Discussion and Conclusion

The study revealed a significant negative correlation between gender and height, weight, arm length, hand quickness scores, and shooting accuracy in basketball players ($p<0.05$, $p<0.01$). Additionally, there was a strong positive correlation found between age and years of sport ($p<0.01$). There were statistically significant positive correlations between height and weight, arm length, hand quickness score, and shooting accuracy score ($p<0.05$, $p<0.01$). Similarly, statistically significant positive correlations between weight and arm length, hand quickness score, and shot accuracy score were found ($p<0.01$). Additionally, a statistically significant positive difference was observed between arm length and hand quickness score and shot accuracy score ($p<0.05$, $p<0.01$). There was a noticeable and significant distinction between hand quickness scores and shooting accuracy scores, with a strong positive correlation ($p<0.01$).

A study by Abdullah (2019) investigated the correlation between vertical jump, shooting, and balance tests and anthropometric factors such as age, weight, sports age, height, and AAHPERD quick shooting test among 24 male and female basketball players in two groups of equal size. The findings revealed a highly significant correlation between shooting and gender, as well as a moderately significant correlation between height and weight. Our study revealed that a negative correlation and a statistically significant gender-based difference were present in relation to shooting accuracy scores ($p<0.01$) [11]. Conversely, no statistically significant difference was found between age and shooting accuracy scores ($p>0.05$).

Professional basketball players strive to attain specific shooting percentages. These goals include 99% for lay-ups, 70% for free throws, 50% for 2-pointers, and 33% or higher for 3-pointers

among professional athletes. These targets may be lower for young basketball players [12]. In our study, we discovered no significant statistical variance concerning shooting accuracy and age ($p>0.05$). The findings suggest that the research groups exhibit a homogeneous structure and demonstrate similar developmental periods when age groups are taken into account.

According to research, the number of years an athlete spends in a particular sport has a linear impact on their performance [13]. Uzun and Pulur (2011) found that basketball players significantly improved their free throw percentage by 106.8% after 10 weeks of training ($p<0.01$), and their accuracy improved in proportion to the duration of the training [14]. Our study found a positive correlation and a statistically significant relationship between the number of years participating in sports, hand quickness scores, and shot accuracy in male athletes. As the athlete's sports history increased, the number of shots taken increased, and shot accuracy improved as expected.

A basketball shooting test was conducted on 24 university students who have actively engaged in basketball for a minimum of 3 years [15]. The participants' upper extremity length, muscle strength, vertical jump reaction time, and Nelson hand reaction time were measured. The group, with an average age of 25, completed a 5-minute warm-up period, after which their dominant hand, total arm length, hand length, forearm, upper arm, and finger lengths were measured in centimetres. Muscle strength, pinch grip, palm grip, and hand grip strength were measured using a hand dynamometer. Reaction time was assessed using the Nelson scale. The study highlights the significance of enhancing free throw performance for achieving match victory. Players exhibited positive results in hand reaction time, vertical jump duration, reaction time, upper extremity extensor strength, and grip strength. After these studies, exercises to improve the upper extremities' coordination, grip strength, and reaction time all contributed to increased shooting accuracy. Our study findings showed a moderate positive correlation between hand quickness score and shot accuracy score in male athletes. However, this relationship did not reach statistical significance ($p<0.05$). A moderate positive and statistically significant difference was found in the data collected from female athletes, between the score for hand quickness and the score for shot accuracy ($p<0.05$).

Shooting is a crucial aspect of basketball. According to Morgan and Dave's (2003) study, shooting accuracy in basketball can be improved by enhancing athletes' self-confidence and training muscle groups responsible for shooting via specially designed regimes [16]. In addition, physical abilities are also essential for a good shooter [14]. A statistically significant positive correlation between weight, arm length and shooting accuracy score ($p < 0.05$) was observed in male athletes

participating in our study. Additionally, a significant positive correlation ($p < 0.01$) was found between height, weight, and arm length of male athletes.

When considering existing literature, Teramoto et al. (2018) investigated the correlation between hand length and 2-point shooting percentage in their analysis of how anthropometric characteristics impact shooting performance, utilizing measurements from the NBA Draft Combine [17]. Following Barut et al.'s (2008) findings, it was concluded that hand length had a significant correlation with two-point shooting performance [18]. However, no significant relationship was found between hand length and 3-point shooting percentage in the same study. The study conducted hand length measurements and analyzed their impact on 2-point shooting results, leading to the observation of a weak correlation between hand length and 2-point shooting percentage. Our study findings indicate a positive and significant correlation between arm length, an anthropometric characteristic of basketball players, and shooting accuracy among male athletes ($p<0.01$). In contrast, the effect of arm length on shooting accuracy was not statistically significant in female athletes ($p>0.05$).

Conclusion

Based on the findings obtained, this study posits that further research investigating the impact of arm length and hand quickness on basketball shooting accuracy could enhance the existing literature. It can be argued that the arm lengths of athletes, particularly with regard to hand quickness, have an impact on shot accuracy. This is a significant factor which coaches must consider when selecting athletes and conducting further research to enhance the reactive abilities of athletes through existing training methods.

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