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ORIGINAL RESEARCH PAPER

Use of Magnetic Nanoparticles to Isolate Anaerobic Bacteria Anaerop Bakterilerin Tanımlanmasında Manyetik Nanopartiküllerin Kullanılması

Received Date: 23.08.2022, Accepted Date: 11.09.2022 **DOI: 10.56484/iamr.1165943**

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Özet:

Giriş: *Normal vücut florasının önemli bir bölümünü oluşturan anaerop bakteriler, yaşamı tehdit eden ciddi enfeksiyonlara yol açabilmektedir. Anaerop bakterilerin izolasyon ve identifikasyonları zaman alıcı ve zor yöntemler gerektirdiği için sadece belli klinik laboratuvarlarda yapılabilmektedir. Bu sebepten anaerop enfeksiyonların tanı ve tedavisi gecikmekte ve ampirik tedaviye bağlı ilaç direnci görülmektedir. Bu bakterilerin erken tanımlanmasını sağlayacak yeni yöntemler, tedavi süresinin ve anaerop enfeksiyonlarına bağlı ölüm oranlarının düşürülmesine yardımcı olacaktır.*

Method: *Bu çalışmada, anaerop bakterilerin hızlı tanımlanmasında kullanılabilecek yeni manyetik nanopartiküllerin tasarlanması amaçlanmıştır. Sentezlenen N - Metil - D - Glukamin (NMDG) bağlı manyetik nanoparçacıklar (Mag-NMDG), geçirimli elektron mikroskopisi (TEM), taramalı elektron mikroskobu (SEM), dinamik ışık saçılımı (DLS), titreşimli örnek manyetometrisi (VSM) ile karekterize edildi.*

Sonuç: *Sentezlenen Mag-NMDG nanopartikülleri, Actinomyces odontolyticus, Prevotella buccae, Veillonella parvula, Bifidobacterium dentium ve Bacteroides fragilis gibi kültür ortamından izole edilen gram pozitif ve gram negatif anaerop bakterilere uygulandı. Bakterilerin Mag-NMDG nanopartiküllere bağlanma durumu mikroskop görüntüleri, McFarland değerleri ve MALDI-TOF MS tanımlama skorları ve spektrumları ile tespit edildi.*

Tartışma: *Bu çalışma neticesinde, geliştirilen Mag-NMDG nanopartiküller anaerop bakterilerin numune ortamından direkt izole edilmesi ve tür düzeyinde tanımlanması için kullanılabileceği belirlendi. Böylece izolasyon ve tanımlama aşamalarındaki zaman alıcı ve zahmetli birçok basamak bertaraf edilebileceği öngörülmektedir.*

Anahtar kelimeler*: Manyetik nanopartiküller, Anaerop Bakteriler, Tanımlama, Bakteri yüzeyinin modifikasyonu*

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Abstract:

Introduction: *Anaerobic bacteria, which make up an important part of normal body flora, may lead to serious life-threatening infections. Since isolation and identification of anaerobic bacteria require time-consuming, sensitive and difficult methods, they can only be performed in certain clinical laboratories. For this reason, diagnosis and treatment of anaerobic infections are delayed and drug resistance is observed due to empirical treatment. New methods that will enable the early identification of these bacteria will help reduce the duration of treatment and mortality rates due to anaerobic infections.*

Method: *In this study, it is aimed to design magnetic nanoparticles attached to N-methyl-D-glucamine (Mag-NMDG) to catch anaerobic bacteria for rapid identification. Mag-NMDG nanoparticles were characterized by transmission electron microscopy (TEM), scanning electron microscopy (SEM), dynamic light scattering (DLS) and vibrating sample magnetometer (VSM).*

Results: *Mag-NMDG nanoparticles were applied to gram positive and gram negative anaerobic bacteria such as Actinomyces odontolyticus, Prevotella buccae, Veillonella parvula, Bifidobacterium dentium and Bacteroides fragilis isolated from culture media. The binding of bacteria to Mag-NMDG was determined by microscope images, McFarland values and MALDI-TOF MS identification scores.*

Conclusion: *As a result of this study, it was concluded that the Mag-NMDG nanoparticles could be used to isolate anaerobic bacteria directly from samples. Thus, it is foreseen that many time-consuming and troublesome steps in the isolation and identification stages can be eliminated.*

Keywords: *Magnetic Nanoparticles, Anaerobic Bacteria, İdentification, Bacteria Surface Modification*

Introduction

Anaerobic bacteria, the majority of which are members of the normal flora, are of great importance for human health. Anaerobic bacteria constitute the majority of the bacterial population in adult humans.[1] Mainly found genera are *Bacteroides, Lactobacillus, Fusobacterium, Bifidobacterium, Eubacterium, Peptococcus, Peptostreptococcus* and *Veillonella.*[2] Bacterial cell wall structures have been extensively studied because they are targets of antibiotics and interact with the human immune system.[3]

The cell walls of bacteria contain a wide variety of glycan structures, including teichoic acids (specific for gram positive organisms), lipopolysaccharides (specific for gram negative organisms), glycolipids, capsule polysaccharides and glycoproteins.[4] It is very difficult to isolate and identify anaerobic bacteria from clinical specimens in the laboratory. Anaerobic bacteria causing polymicrobial infections and accompanying secondary infections make it difficult to isolate and identify these bacteria from clinical samples. As a result, it makes the choice of antibiotics to be used against these bacteria seriously uncertain.[4] Accurate and rapid identification of anaerobic bacteria is important for the correct diagnosis, treatment and follow-up of the disease.

Today, many different methods are used to identify anaerobic bacteria. These methods are generally based on in vitro cultivation, morphological and biochemical analysis. However, these methods, which are used to obtain and identify bacteria purely, involve many sensitive and laborious steps that can last up to 3-4 days and are insufficient to identify certain species.[5,6] Besides, PCR, ribotyping, ELISA, microarray etc. molecular-based diagnostic methods are also available. Although the sensitivity and specificity of these methods are high, they have disadvantages such as high cost, trained personnel and intensive sample pre-treatment. These disadvantages of traditional and molecular-based methods can be overcome by using nanotechnological methods.[7,8] Nanotechnology is a low-cost technology that requires few samples and provides reliable results in a very short time.[9] Magnetic iron oxide nanoparticles produced with this technology have natural advantages such as ease of surface modification, precisely controllable sizes, and large surface areas.[9] Therefore, the use of nanotechnology in clinical diagnostic methods is gaining importance day by day. However, in order to use nanotechnological diagnostic methods as a real alternative to clinical diagnostic methods, various modifications should be made to these particles at the molecular level and the sensitivity and specificity of the method should be increased.[10,11]

The aim of this study is to rapidly isolate and identify anaerobic bacteria with N-Methyl-D-Glucamine (NMDG) attached magnetic nanoparticles (Mag-NMDG). For this purpose, magnetic nanoparticles were functionalized with NMDG and characterized by transmission electron microscopy (TEM), scanning electron microscopy (SEM), dynamic light scattering (DLS) and vibrating sample magnetometer (VSM). The Mag-NMDG nanoparticles could be used to isolate anaerobic bacteria directly from samples. Subsequently, Mag-NMDG nanoparticles were used in isolation and identification of anaerobic bacteria from samples.

Materials and Methods

1. Materials

Ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), N-methyl-D-glucamine ($C_7H_{17}NO_5$), Ferrous chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$), 3-bromopropyl trimethoxysilane ($C_6H_{15}BrO_3Si$), Toluene, anhydrous| ($C_6H_5CH_3$) and Ethanol (C_2H_5OH) were purchased from Sigma-Aldrich and all of them were of analytical grade. Different solutions at various concentrations used in different experiments were gained by dilution of the stock solution. Every reagent that used were of analytic grade and used as such.

2. Synthesis and Characterization of Mag-NMDG Nanoparticles

The Mag-NMDG nanoparticles was synthesized by a three-step procedure. In the first step, magnetic Fe_3O_4 nanoparticles were fabricated by co-precipitation method reported in literatüre.[12] In the second step, the surface of the magnetic Fe_3O_4 nanoparticles coated with a (3-Bomopropyl) trimethoxysilane by some modifications. Briefly, 1 g of magnetic Fe_3O_4 nanoparticles powder was dispersed in 25 mL of dry toluene by sonicator for 30 min. Then, 0,5 mL (3-Bomopropyl) trimethoxysilane was added and the solution was refluxed at 60 °C and for 18 h.

Fe_3O_4@SiO_2-Br was washed with toluene several times, then separated by using neodymium magnet, final product dried by vacuum freeze dryer.[13,14] In final step, the obtained Fe_3O_4@SiO_2-Br (1 g) as supported was dispersed in 10 mL of water, and then NMDG (0.780 g) was added to the reaction mixture and this mixtrure was refluxed for 24 h. finally Mag-NMDG separated by magnetic decantaion and washed with deionized water to remove the unreacted chemicals and substances and then dried on a freeze dryer.[15,16]. Reaction sequences of synthesis for Mag-NMDG are shown in Figure1.

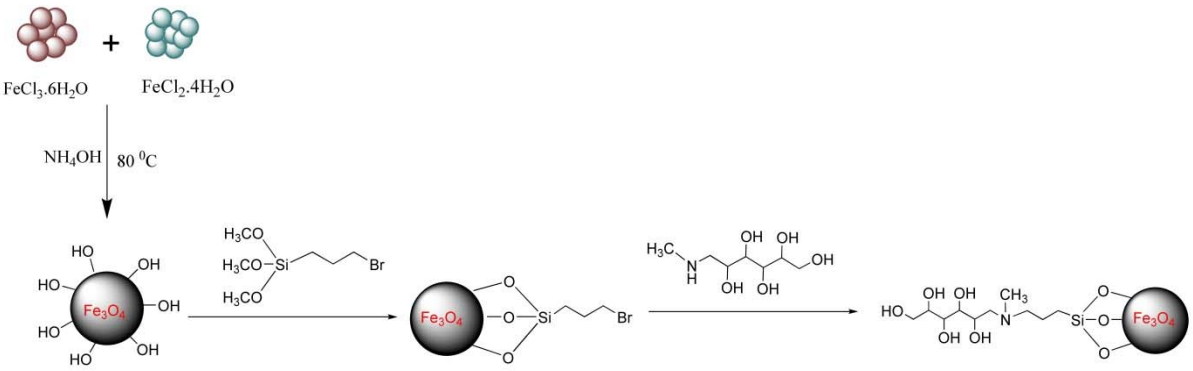

Figure 1. *The synthesis mechanism of Mag-NMDG nanoparticles*

3. Sample Cultivation, Bacteria Isolation and Identification

Samples requested for anaerobic culture from various clinics were sent to the bacteriology laboratory via sterile glass tubes, sterile plastic containers or sterile syringes. All clinical samples were inoculated onto anaerobic blood agar (BD *Brucella* Agar 5% Sheep Blood, 1 mg/L vitamin K1, 5 mg/L hemin, Becton Dickinson, Franklin Lakes, NY) and thioglycolate broth (BD Fluid Thioglycolate Medium, Becton Dickinson), incubating all media at 35-37 °C for 5 days.

Gram staining of all isolated anaerobic bacteria species was performed and images were taken under a light microscope. In addition, identification of all isolates was carried out using MALDI-TOF MS (Bruker Biotyper, Bellerica, MA, USA). Identified bacterial species are: *Bacteroides fragilis, Prevotella buccae, Veillonella parvula, Bifidobacterium dentium, Actinomyces odontolyticus.*

4. Detection of Anaerobic Bacteria Using Mag-NMDG Nanoparticles

McFarland, Gram stain and MALDI-TOF MS methods were used for the determination of bacterial isolation with Mag-NMDG. The isolation and detection stages of anaerobic bacteria with Mag-NMDG nanoparticle are shown in Figure 2.

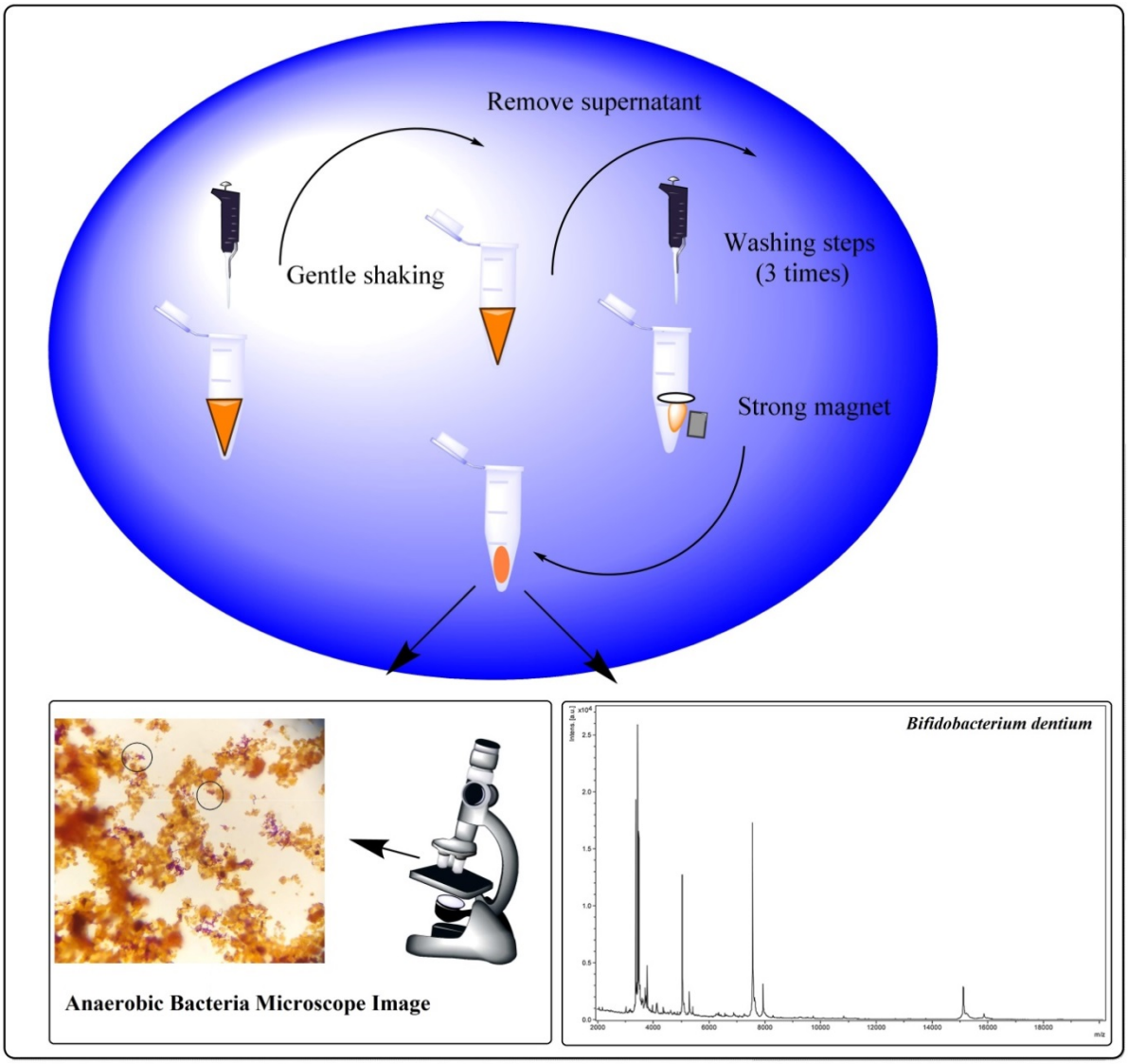

Figure 2. *Isolation and Detection Stages of Anaerobic Bacteria Using Mag-NMDG Nanoparticle*

5. Ethical Procedures

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

Result

1. Characterization of Mag-NMDG Nanoparticles

The magnetic properties of Mag-NMDG nanoparticle was researched by VSM analysis at room temperature. The saturation magnetization of Mag-NMDG was measured as approximately 38.2 emu/g (Figure 2A). The saturation magnetization value of Mag-NMDG is suitable for magnetic separation. Agglomerate size states of the synthesized Mag-NMDG nanoparticles were analyzed by DLS by providing dispersions in water. For the reliability of the results, each sample was distributed in the sonicator prior to the analysis was performed. Figure 2B shows the agglomeration size of Fe_3O_4, Fe_3O_4@SiO_2-Br and Mag-NMDG by DLS. As shown in Figure 2B, the particle sizes of Fe3O4, Fe_3O_4@SiO_2-Br and Mag-NMDG nanoparticles were measured to be approximately 141, 479 and 724 nm respectively.

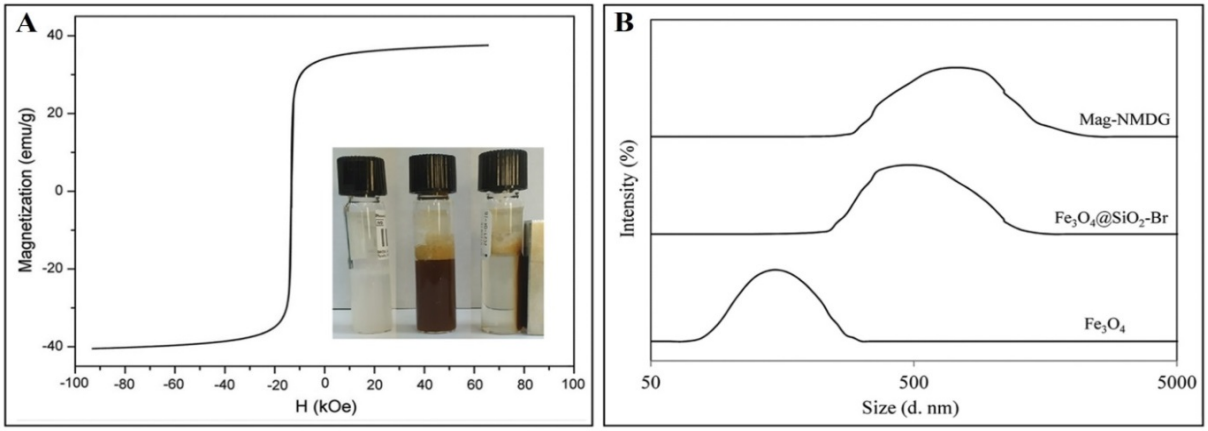

Figure 3. *(A) Magnetization curve of Mag-NMDG against magnetic field, (B) Size distribution graph of Fe_3O_4, Fe_3O_4@SiO_2-Br and Mag-NMDG nanoparticles*

TEM image of Mag-NMDG is given in Figure 3A. The size and shape analysis of the synthesized Mag-NMDG nanoparticles were analyzed with the TEM device. The nanoparticle size of Mag-NMDG was determined to vary between 12-16 nm.

SEM image of Mag-NMDG is given in Figure 3B. This image contains similar spherical images confirming that the surface morphology of Mag-NMDG nanoparticles is similar. These findings are consistent with DLS analysis results.

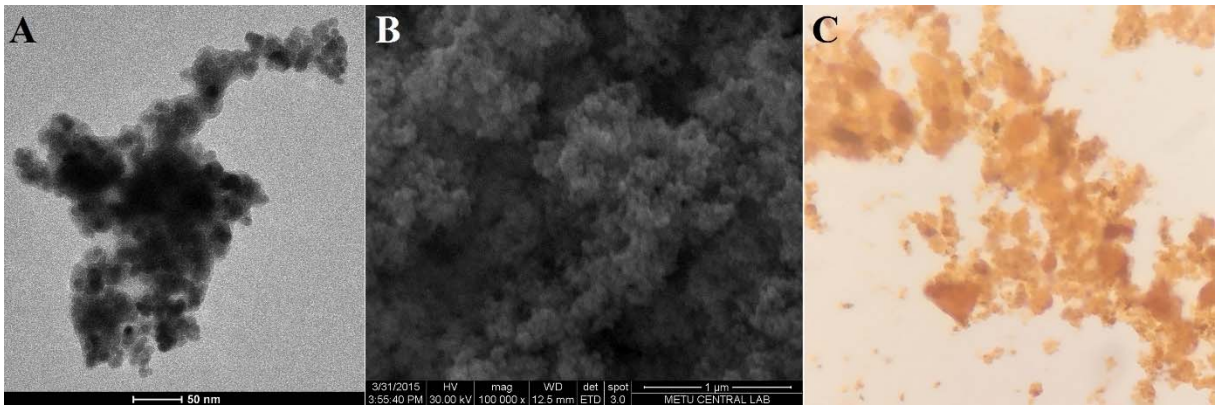

Figure 4. *(A) TEM image and (B) SEM image C)* **Optical microscopy images** *of Mag-NMDG nanoparticles*

2. Detection by McFarland Method

The McFarland turbidity standard value of bacteria grown in culture was set to 1.0 (3×10^8 cfu/ mL) and the results were given (Table 1). 4 mL of liquid was taken from the samples whose McFarland turbidity values were adjusted as 1.0 (3×10^8 cfu/mL) and transferred to tubes containing 10 mg Mag-NMDG. In order to occur the Mag-NMDG-bacteria complex, the tubes were vortexed for 5 minutes and kept at room temperature for 30 minutes. After the time, the Mag-NMDG-Bacteria complex was precipitated with the aid of a magnet. After magnetic separation was completed, the supernatant was transferred to another tube, McFarland values were determined, and the results were given in Table 1.

3. Detection by MALDI-TOF MS

The MALDI-TOF MS method was used to investigate whether the Mag-NMDG method could be used for species identification. First, the identification of anaerobic bacteria grown in culture was done with MALDI-TOF MS (Table 1). The same bacteria were then treated with Mag-NMDG nanoparticles. The bacterial species in the bacteria-Mag-NMDG complex taken from the pellet formed after the magnetic separation process were again identified by MALDI-TOF MS and the obtained scores were given (Table 1). Mass spectra obtained after identification with MALDI-TOF MS are shown in Figure 5. In our study, it was observed that the MALDI-TOF MS identification scores obtained before and after the experiment as a result of the treatment of isolated bacteria with Mag-NMDG nanoparticles were very close to each other. This shows that Mag-NMDG nanoparticles can be used together with the MALDI-TOF MS method for the identification of anaerobic bacteria.

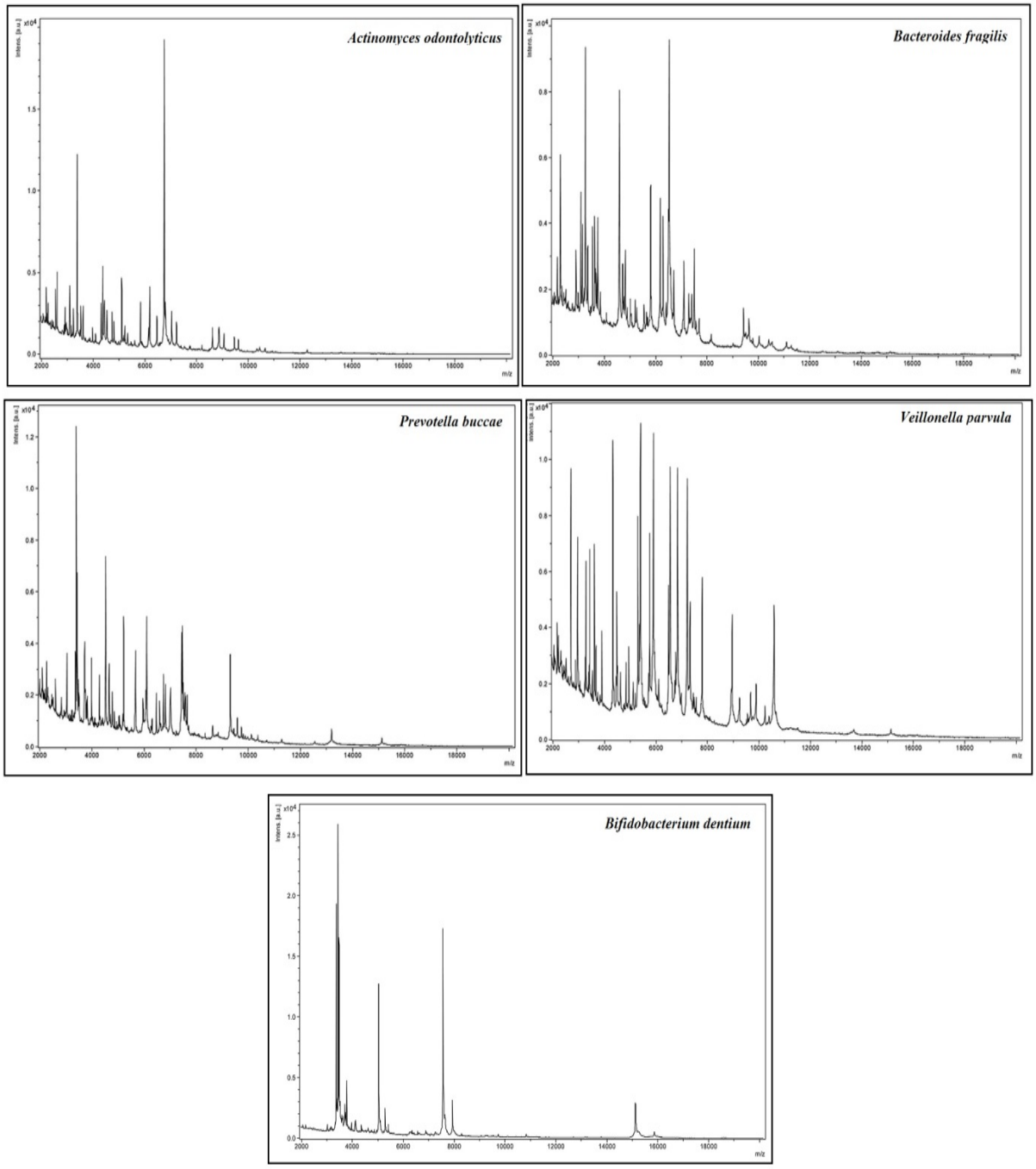

Figure 5*. Mass spectra of the Bacteria-Mag-NMDG complex obtained by MALDI-TOF MS*

4. Detection by Gram Stain

Gram staining was performed from the pellet to view the bacteria-Mag-NMDG complex formed after the magnet separation, and the formation of the bacteria-Mag-NMDG complex was observed under the light microscope (Figure 6, Figure 7, Figure 8, Figure 9, Figure 10).

Table 1: *Mcfarland and MALDI TOF MS Results of Anaerobic Bacteria Before and After the Experiment*

Anaerobic Bacteria	MALDI TOF MS		McFarland	
	Before Experiment	After Experiment Pellet	Before Experiment	After Experiment Supernatant
Bacteroides fragilis	2.304	1.831	1.08	0.81
Prevotella buccae	2.017	1.905	0.98	0.61
Veillonella parvula	2.320	1.827	1.04	0.64
Bifidobacterium dentium	1.901	1.841	1.01	0.53
Actinomyces odontolyticus	2.050	1.990	1.04	0.46

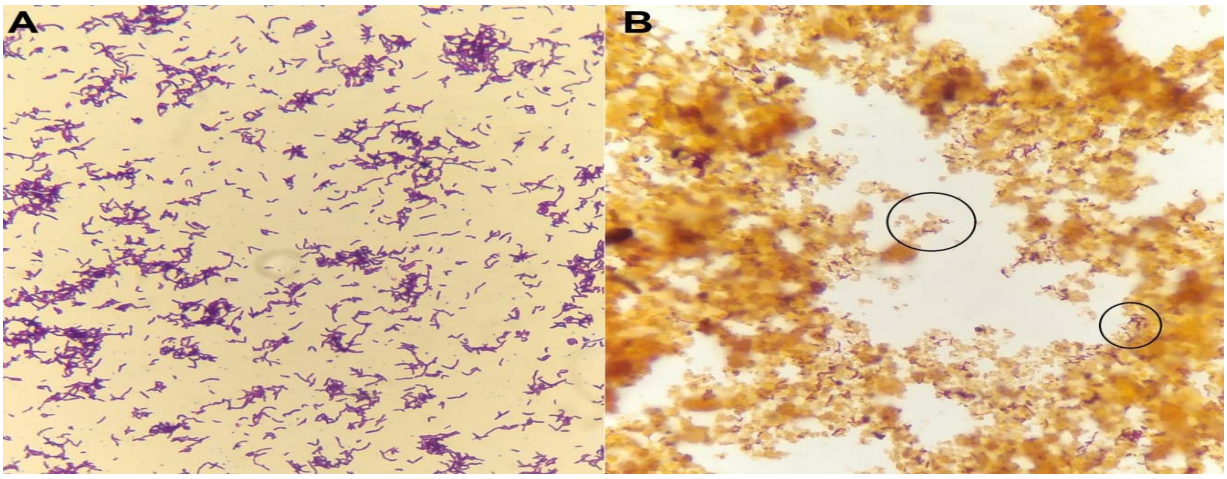

Figure 6. *(A) Actinomyces odontolyticus, (B) Actinomyces odontolyticus- Mag-NMDG*

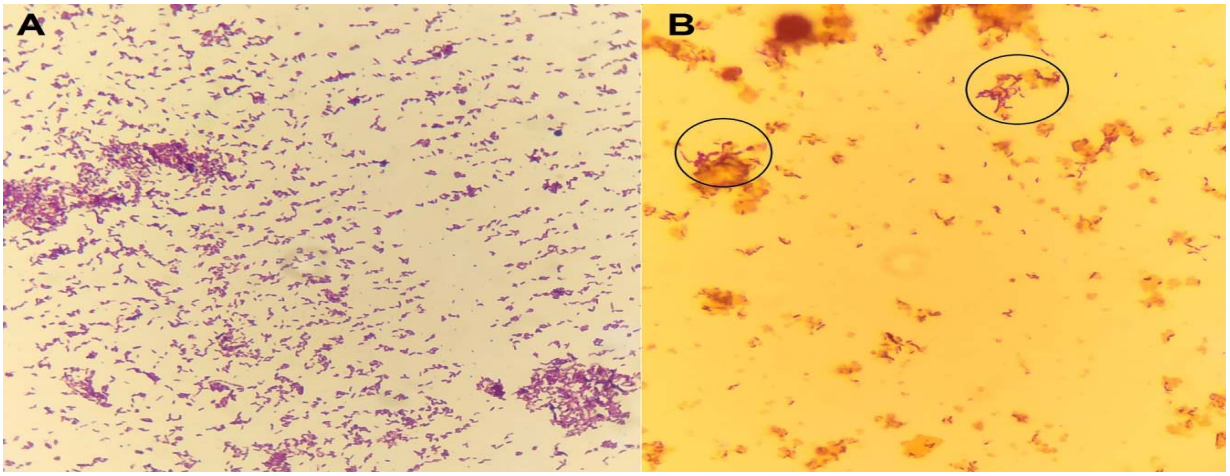

Figure 7. *(A) Bifidobacterium dentium, (B) Bifidobacterium dentium- Mag-NMDG*

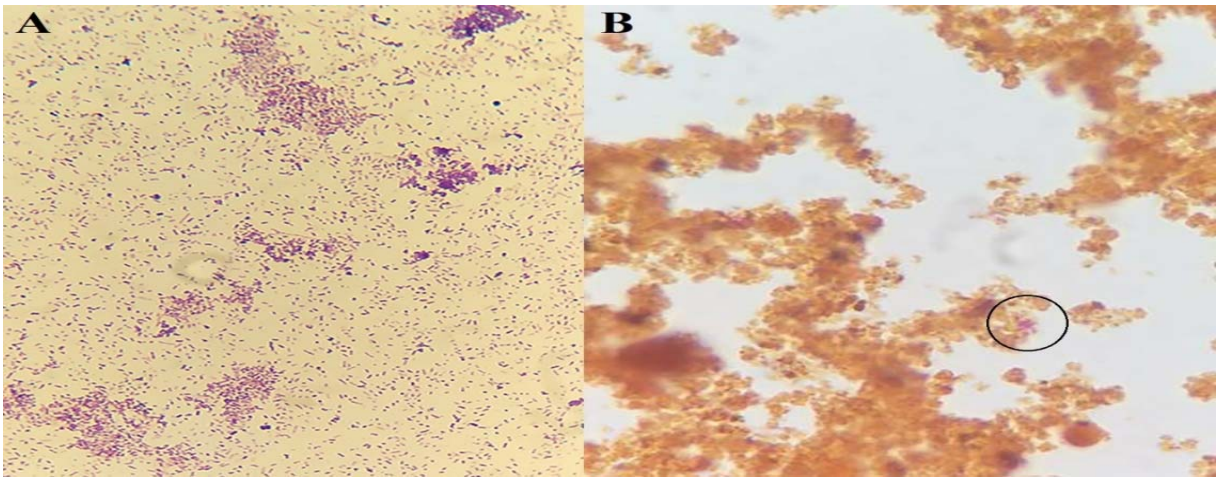

Figure 8. *(A) Prevotella buccae, (B) Prevotella buccae - Mag-NMDG*

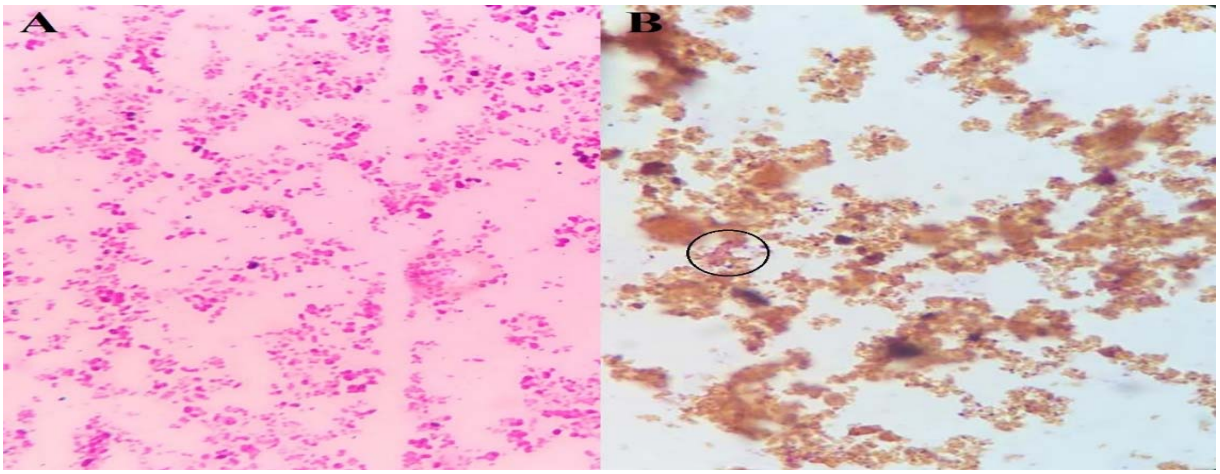

Figure 9. *(A) Veillonella parvula, (B) Veillonella parvula - Mag-NMDG*

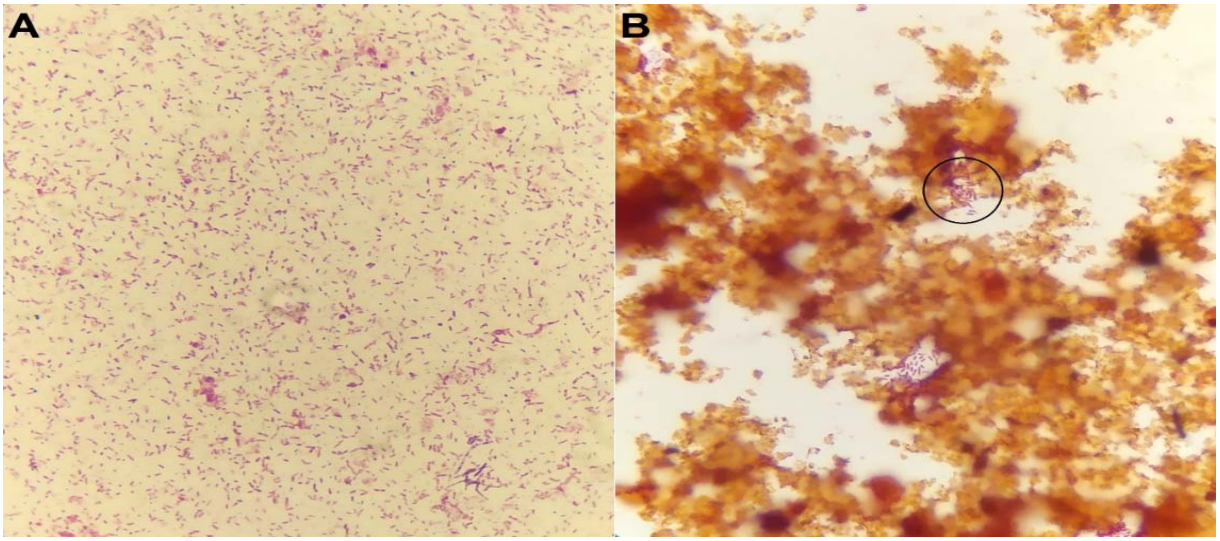

Figure 10. *(A) Bacteroides fragilis, (B) Bacteroides fragilis - Mag-NMDG*

Conclusion

Magnetic nanoparticles have the ability to bind to biological structures. Therefore, it has great potential in the detection of bacteria. Magnetic nanoparticles formed in appropriate proportions provide the opportunity to catch bacteria and isolate them from the environment. This approach has provided an attractive and innovative method for bacterial diagnostic methods.[5,6] The cell walls of bacteria contain a wide variety of glycan structures, including teichoic acids (specific to gram-positive organisms), lipopolysaccharides (specific to gram-negative organisms), glycolipids, capsule polysaccharides, and glycoproteins. These structures contain some functional groups such as (–COO−), (–NH) , (–OH), (–C=O), (–C–N–), (–C–O), (–C–H). Each of these groups has different affinity and adsorbs different molecules.[13] Studies on the use of nanoparticles in the detection of bacteria are increasing day by day. Suaifan et al[17]. measured the color change based on proteolytic enzyme activity of *Staphylococcus aureus* using magnetic nano beads and thus made quantitative analysis of the bacteria in the sample. In the study of Tural et al[13]., they successfully prepared a new magnetic biosorbent by immobilizing *Bacillus subtilis* with nano-sized magnetic silica. Thus, they used this biosorbent to remove methylene blue pollution. Gautam et al[3]. showed in detail how bacterial cell surface structures can be modified with functional groups on different bacterial species in their study. All these studies show that bacteria-specific components such as enzymes and cell wall structures can make specific bonds with nanoparticles, and this complex can be used in many different areas, including bacterial identification.

Therefore, the formation of the bacteria - Mag-NMDG complex appears to depend on the affinity of these functional groups. In our study, it was observed that magnetic nanoparticles functionalized with NMDG can bind to the cell wall structures of Gram-negative and Gram-positive anaerobic bacteria and can thus be isolated from samples. As a result, as seen in Table 1 in our study, the McFarland and MALDI TOF MS values of Gram-negative and Gram-positive anaerobic bacteria isolated by traditional methods before and after the experiment also show that the functional groups can combine with these bacterial groups under appropriate conditions.

According to McFarland results, Mag-NMDG nanoparticles provided high adhesion to all bacterial species in our study. The pellet was transferred to the MALDI TOF MS device for species identification. MALDI TOF MS identification scores were determined at the species level. In addition, the formation of the bacteria-Mag-NMDG complex is shown by the optical microscope images given in Figures 5, 6, 7, 8 and 9. These results obtained in our study show that the nanoparticles we have developed can be used for the isolation and identification of certain anaerobic bacteria from the samples.

As a result; we succeeded in attaching the Mag-NMDG nanoparticles we had developed to anaerobic bacteria such as *Bacteroides fragilis, Prevotella buccae, Veillonella parvula, Bifidobacterium dentium, Actinomyces odontolyticus.*

We demonstrated that Mag-NMDG nanoparticles can be used in a collaborative manner with MALDI-TOF MS to identify anaerobic bacteria at the species level. Since our research is a proof of concept for Mag-NMDG, specificity / cross-reactivity experiments have not been conducted, but these will be part of future studies. With this study we have done, nanotechnology; we believe that developing powerful combinations with existing techniques for the early and rapid detection of bacteria in samples will become more sensitive and cost-effective than current laboratory techniques.

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ORIGINAL RESEARCH PAPER

Analysis of Hand, Face, and Body Contact Dermatitis in Children with Chemical Patch Tests

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Abstract:

Objective: *Allergic contact dermatitis is a late-type (type IV) hypersensitivity reaction that occurs after repeated exposure to previously sensitized substances. There are 3700 chemicals reported that can cause this condition. There are publications in the literature indicating that the hands and face are the most frequently involved body parts in contact dermatitis in adults. However, since there is insufficient data on which region is more frequently involved in children and allergen examination according to the involved areas, this study was aimed to be conducted.*

Materials and Methods: *In our study, 102 patients who applied to our outpatient clinic between January 2019 and June 2021 with a diagnosis of contact dermatitis and underwent a chemical patch test were included and retrospectively analyzed.*

Findings: *Of the 102 patients included in the study, 43 were female (42.2%), and 59 were male (57.8%). The ages of the patients ranged from 4-18 years, with a mean age of 9.45±3.92 years. When the body parts with contact dermatitis were scrutinized, 33 (32.4%) had hand involvement, 7 (6.9%) had hand and arm involvement together, and 2 (2%) had hand and face involvement together. Facial involvement was present in 19 patients (18.6%), and 5 patients (4.9%) had involvement of the body and face together. Body involvement was identified in 36 patients (35.3%).*

Result: *When Myroxylon Pereiare Resin and Formaldehyde sensitizers were evaluated according to lesion localization, the difference between them was statistically significant ($p<0.05$). Myroxylon Pereiare Resin was identified positive in 8 of the patients with body contact dermatitis. Formaldehyde sensitization was observed in 5 of 19 patients with facial involvement. There was no significant difference between lesion localization and positivity rates in other chemical parameters.*

Key Words: *Allergic contact dermatitis, Patch test, Myroxylon Pereiare Resin, Formaldehyde*

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Introduction

Contact dermatitis is an inflammatory condition caused by direct contact with a specific chemical [1]. Irritant contact dermatitis is a non-specific inflammatory cutaneous reaction to an irritant substance. Allergic contact dermatitis (ACD), on the other hand, is a late-type (type IV) hypersensitivity reaction that occurs after re-exposure to previously encountered substances to which sensitization has developed [2]. While it may cause symptoms such as erythema, edema, vesicle, and intense pruritus in acute stages, hyperkeratosis, desquamation and lichenification may develop when it becomes chronic [3]. Metals, cosmetics, fragrances, preservatives, textiles, rubber, resins, plants, topical treatments, and many other allergens can cause contact dermatitis. The most common causative substances vary according to the frequency of use of allergens and geographical regions [4]. 3700 chemical substances that may cause ACD have been reported [5].

The diagnostic criteria for Allergic Contact Dermatitis are patient history (presence of substance contact), physical examination (allergic lesions) and skin patch test. Patch testing is the most important method used to confirm the diagnosis in patients. The positivity of the test may vary according to age, gender, presence of atopy, the series used and the characteristics of countries and regions [6]. In 2018, a study published in Europe reported that allergic contact dermatitis affects 27% of the general population [7].

The increase in the number of published studies on patch test results in children and adolescents has contributed to the vast body of knowledge on the subject, demonstrating that contact sensitization and allergic contact dermatitis are not uncommon in childhood or adolescence [8].

Aim: There are publications in the literature indicating that the hands and face are the most frequently involved body parts in contact dermatitis in adults. However, since there is insufficient data on which region is more frequently involved in children and allergen examination according to the involved areas, this study was aimed to be conducted.

Materials and Methods

Between January 2019 and June 2021, 102 patients admitted to our outpatient clinic with a diagnosis of contact dermatitis who underwent chemical patch testing were retrospectively analyzed. Age, gender, lesion localizations, results of chemical patch tests, eosinophil count, percentage, and total IgE levels were recorded in patients whose laboratory tests were obtained on admission.

All patients underwent patch testing with chemotechnique MB© standard series. It was taken into consideration that the patients did not have active lesions at the time of patch test application

and had not used topical corticosteroids and immunosuppressive drugs in the last week before the test. Allergens were attached to the upper back of the patients using IQ Chambers© (Chemotechnique Diagnostics, Sweden). The tests were evaluated after opening after 48 hours and waiting for 30 minutes. Tests were evaluated for the second time at 96 hours and for the third time on day 7. The evaluation was performed according to the criteria determined by the International Contact Dermatitis Research Group [9].

Statistical Analysis

Data analysis was performed with IBM SPSS 21 package program. Summary values for qualitative variables were given as frequency and percentage, and for quantitative variables as mean ± standard deviation and median (Q1-Q3). The relationship between patch test results and other qualitative variables was evaluated by chi-square analysis (Pearson, Yates, Fisher Exact). The conformity of quantitative variables to normal distribution was evaluated by the Shapiro Wilk test. Comparison of non-normally distributed blood parameters according to lesion localization regions was performed by the Kruskal Wallis test. Cases with a $p<0.05$ analysis result were considered significant.

Result

Of the 102 patients included in the study, 43 were female (42.2%) and 59 were male (57.8%). The ages of the patients ranged from 4-18 years, with a mean age of 9.45±3.92 years. Some patients had contact dermatitis on more than one body part.

When the body parts with contact dermatitis were scrutinized, 33 (32.4%) had hand involvement, 7 (6.9%) had hand and arm involvement together, and 2 (2%) had hand and face involvement together. Facial involvement was present in 19 patients (18.6%), and 5 patients (4.9%) had involvement of the body and face together. Body involvement was detected in 36 patients (35.3%) (Table 1).

Table 1. Distribution of patients according to lesion localizations

Lesion localizations	Number of patients (%)
Hand	33 (%32,4)
Face	19 (%18,6)
Body	36 (%35,3)
Hand+ Face	2 (%1,96)
Hand+ Arm	7 (%6,9)
Body+ Face	5 (%4,9)
Total	102 (%100)

When the patients who showed positive reactions in the patch test results were separated according to gender, the number of sensitization in male and female patients was twenty to ten for Potassium Dichromate, ten to two for Myroxylon Pereiare Resin, and eighteen to seven for Fragrance Mix. However, since the number of patients was not sufficient, there was no statistically significant difference between genders in terms of allergens with positive reactions (P>0.05) (Table 2).

Table 2: Distribution of patients with positive skin patch test results by gender

Allergen	Male (%)	Female (%)	P
Potassıum Dichromate	17 (%28,8)	13 (%30,2)	1,00
Phenylenediamine Base	5 (%8,5)	2 (%4,7)	0,69
Neomycin Sulfate	14 (%23,7)	13 (%30,2)	0,61
Cobalt(2) Chlorıde Nexahydrate	22 (%37,3)	22 (%51,2)	0,23
Benzocaine	3 (%5,1)	2 (%4,7)	1,00
Nickelsulfate Hexahydrate	14 (%23,7)	13 (%30,2)	0,61
Cliguinol	0	2 (%4,7)	0,17
Colophony	6 (%10,2)	2 (%4,7)	0,46
Paraben Mix	20 (%33,9)	10 (%23,3)	0,34
N-Isopropyl-N-Phenyl-4-Phenylenediamine	7 (%11,9)	5 (%11,6)	1,00
Lanolin Alcohol	15 (%25,4)	7 (%16,3)	0,38
Epoxy Resin	6 (%10,2)	3 (%7,0)	0,73
Myroxylon Pereiare Resin	10 (%16,9)	2 (%4,7)	0,11
4-Tert Butylphenolformaldehyde Resin	2 (%3,4)	1 (%2,3)	1,00
Mercaptobenzothiazote	3 (%5,1)	5 (%11,6)	0,27
Formaldehyde	5 (%8,5)	5 (%11,6)	0,73
Fragrance Mix	18 (%30,5)	7 (%16,3)	0,15
Sesquiterpenelactone Mix.	2 (%3,4)	3 (%7,0)	0,64
Quaternium-15	3 (%5,1)	0	0,26
2-Mexhoxy-6-N-Pentyl-4-Benzoquinone(Primin)	4 (%6,8)	4 (%9,3)	0,71
Methylisothiazolinone+ Methychloroisothiazolinone	8 (%13,6)	8 (%18,6)	0,67
Budesonide	0	3 (%7,0)	0,07
Tixocortol-21-Pivalate (Mdbgn)	3 (%5,1)	2 (%4,7)	1,00
Methyldibromo Glutaronitrile	3 (%5,1)	3 (%7,0)	1,00
Fragfance Mixll	6 (%10,2)	3 (%7,0)	0,73
Methylisothiazolinone	9 (%15,3)	5 (%11,6)	0,81
Textile Dye MixLyral	6 (%10,2)	5 (%11,6)	1,00

In our study, 84 (82.3%) of 102 patients who underwent patch testing were positive for one or more of the allergens tested. Eight patients had a positive reaction to one allergen, while in 76 patients (74.5%) positivity to more than one allergen was detected. Most common allergens to which reactions were detected: Cobalt(2) Chloride Nexahydrate (43.1%), Potassium Dichromate (29.4%), Paraben Mix (29.4%), Neomycin Sulfate (26.5%), Nickel Sulfate (26.5%), Fragrance Mix (24.5%),

Lanolin (21.6%), Methylisothiazolinone+ Methychloroisothiazolinone (15.7%), Methylisothiazolinone (13.7%), Myroxylon Pereiare Resin (11.8%), Textile Dye MixLyral (10.8%). These parameters were also evaluated in patients whose IgE and hemogram tests were taken at the time of admission. The minimum, maximum and median values of eosinophil counts, eosinophil percentages, and IgE values were compared according to lesion localizations, and the difference between them was not statistically significant (P>0.05) (Table 3).

Table 3: Distribution of blood values according to lesion localizations

Blood Parameters	Hand	Face	Body	P
Ig E Mean(Min-Max)	140,94±187,39 69,5(26-227)	492,25±738,75 130,5(23-854,5)	97,36±110,8 63,5(21-116)	0,67
Eosinophil count Mean(Min-Max)	298,15±218,1 270(140-440)	252,5±208,42 155(90-460)	297,89±214,78 220(140-400)	0,73
Eosinophil Mean(Min-Max)	3,77±2,54 3(1,7-4,7)	3,55±2,39 2,7(1,6-6)	4,17±3,17 2,8(1,9-6,3)	0,93

Patients with hand, face, and body contact dermatitis were grouped and analyzed according to the allergens found positive (Table 4). While grouping, patients with body and face involvement together and patients with hand and face involvement together were excluded. Those with hand and arm involvement were analyzed in hand contact dermatitis. Allergens causing contact dermatitis on the hands, face, and body were compared according to the localizations of involvement and the p value was calculated. Myroxylon Pereiare Resin was positive in 8 patients with body contact dermatitis, 2 patients with hand contact dermatitis, and 1 patient with facial contact dermatitis, and the difference between these values was statistically significant (p=0.036936). A total of 10 patients with hand, face, and body contact dermatitis developed sensitization with Formaldehyde; 5 of these patients had facial contact dermatitis, and only one patient had body localized contact dermatitis, and the difference between the results was statistically significant (p=0.021625). There was no significant difference between lesion localization and positivity rates in other chemical parameters.

Table 4: Distribution of patch test results according to lesion localization

Allergen	Hand	Face	Body	P
Potassıum Dichromate	11	4	11	0,75
Phenylenediamine Base	2	1	2	1,00
Neomycin Sulfate	10	3	12	0,36
Cobalt(2) Chlorıde Nexahydrate	19	6	16	0,50
Benzocaine	2	0	2	0,68
Nickelsulfate Hexahydrate	10	3	10	0,60
Cliguinol	1	1	0	0,67
Colophony	5	0	3	0,28
Paraben Mix	11	9	8	0,14
N-Isopropyl-N-Phenyl-4-Phenylenediamine	3	1	7	0,16
Lanolin Alcohol	8	3	8	0,85
Epoxy Resin	3	3	3	0,59
Myroxylon Pereiare Resin	2	1	8	***0,03***
4-Tert Butylphenolformaldehyde Resin	0	0	2	0,17
Mercaptobenzothiazote	2	2	4	0,62
Formaldehyde	4	5	1	***0,02***
Fragrance Mix	10	5	8	0,93
Sesquiterpenelactone Mix.	1	1	3	0,72
Quaternium-15	0	1	1	0,50
2-Mexhoxy-6-N-Pentyl-4-Benzoquinone(Primin)	4	2	2	0,79
Methylisothiazolinone+ Methychloroisothiazolinone	6	2	7	0,67
Budesonide	1	1	0	0,68
Tixocortol-21-Pivalate (Mdbgn)	1	1	3	0,72
Methyldibromo Glutaronitrile	4	0	2	0,36
Fragfance Mixll	2	2	4	0,62
Methylisothiazolinone	6	2	5	0,93
Textile Dye MixLyral	6	3	1	0,13
Gold sodıum thiosulfate	2	0	0	0,35
Wool alcohols	1	0	0	1,00

Discussion

Although a detailed history is the first step in diagnosing allergic contact dermatitis, patch testing is the most valuable method that allows us to confirm the diagnosis and identify the allergen. Although this test is more than 100 years old, it is still a diagnostic approach that provides a high degree of standardization and was developed by Jadassohn in 1895 [10,11].

Studies evaluating the prevalence of ACD in children have reported patch test positivity rates between 14.5-70.7% [12,13]. In studies conducted in our country, the frequency of positive reactions in patch tests was reported to vary between 31.3% and 73.75% (14). In our study, 84 (82.3%) of 102 patients who underwent patch testing were positive for one or more of the allergens tested.

In a study conducted by Seidenari S et al. in children between the ages of 7 months and 12 years, it was reported that the highest sensitivity was found under the age of three[14]. Wöhrl S. et al. reported that the overall sensitivity rate decreased continuously, being highest in children younger than 10 years (62%) and lowest in patients older than 70 years (34.9%) [15]. There is no consensus among different studies on the effect of age on the incidence of ACD in children, but in the study by (Alt+1) Hammonds LM et al. [16], it was shown that in younger children (<10 years), males were more likely to have a positive patch test. We think that sensitization may develop earlier in children with the increasing use of chemicals and the increasing diversity in care products and that this is reflected in the positivity rates of our study.

In a study conducted in 2009, it was reported that there was no significant difference between different age groups (3-10, 11-15, 16-18 years), although there was a tendency to respond less positively with increasing age in males [17]. In our study, no statistically significant difference was found when the age distribution of patients with positive patch test was evaluated.

Of the 102 patients in our study, 43 were female (42.2%) and 59 were male (57.8%). There was no statistically significant difference between sexes regarding sensitization rate in patch tests. However, in an analysis published in 2015, which examined patients between 2002 and 2010 across Europe, it was reported that no difference was found between boys and girls in terms of susceptibility [18].

There are many publications in the literature on contact dermatitis and patch tests. The aim of our study was to determine whether the allergens reacted to differ according to body parts and which substances cause more sensitization. In a study conducted for this purpose and published in 2019, it was shown that contact dermatitis on the hands was more common, although the majority of patients were aged 40 and over and different results were obtained in different countries. In addition, when allergic contact dermatitis of the hands and feet were compared in this study, methylisothiazolinone was found to be a common contact allergen in patients with ACD of the hands and thiuram mixture was a common contact allergen in patients with ACD of the hands, while mercapto mixture and 2-mercaptobenzothiazole were common in patients with ACD of the feet. While Myroxylon pereirae, colophonium, lanolin alcohol, and paraben mixture were contact allergens frequently found in patients with leg ACD, positive patch test reactions to N- isopropyl- N' -phenyl- p-phenylenediamine were common in patients with head and upper extremity ACD, but not in patients with body and lower extremity ACD [19].

In our study, Myroxylon Pereiare Resin was found to have a statistically higher positive rate in patients with body ACD compared to hand and face contact dermatitis. Myroxylon Pereiare Resin is included in cosmetics, personal care products, and some topical formulations.

In addition, in our study, Formaldehyde was found to be statistically significantly higher in patients with facial ACD compared to other regions. Formaldehyde is generally found in cosmetic products, creams, shampoos and soaps [20].

In a study published by Tunca M. et al. in 2019, patch test results were examined and it was reported that the most common positive allergen was Nickel Sulfate with 18.3% [21]. However, the most frequently detected allergens in our study were Cobalt (2) Chloride Nexahydrate (43.1%), Potassium Dichromate (29.4%), Paraben Mix (29.4%).

Since our study was single centered and included only pediatric ACD cases, the number of patients and the number of sensitized allergens were limited. Therefore, although differences were found with some allergens according to the sites of involvement, statistically significant differences could not be obtained. Physicians and families in our society should be informed about ACD and patients should be referred to physicians and clinics specialized in contact dermatitis where patch tests can be performed. In addition, the allergens sensitized in ACD may vary according to geographical conditions, lifestyle and the variety of products used in daily life. Therefore, there is a need for multi-centered studies across Turkey. In our country, there is also insufficient data on allergens sensitized according to the areas of contact dermatitis involvement in children. For this purpose, studies with more patient participation should be carried out by grouping according to the regions where dermatitis is seen.

Conclusions

When the lesion location was investigated, the difference between the sensitized ones with Myroxylon Pereiare Resin and Formaldehyde was shown to be statistically significant (p0.05). Myroxylon Pereiare Resin was found to be positive in 8 of 36 people with body-only contact dermatitis. Sensitization developed in five of the 19 individuals who had formaldehyde in their faces. In addition, because allergies vary based on regional conditions and the drugs used, multicenter study across Turkey should be undertaken.

Acknowledgments

Potential for conflict of interest.

There are no conflicts of interest declared by the authors.

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ORIGINAL RESEARCH PAPER

Cytoxic Effect Levels Of Sheep Whey Protein in Colorectal Adenocarcinoma Cell Line (Caco-2)

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Abstract:

Objective: *It was aimed to determine the antitumor and antiproliferative activity of whey protein (whey) obtained from sheep colostrum isolation on colorectal cancer cells (Caco-2).*

Method: *Colostrum was separated into fractions as whey proteins using the isoelectric point. Before cell culture was studied, whey protein was sterilized by a membrane filter. Antitumoral activity levels of different concentrations of lyophilized proteins were measured by MTT viability test on cells and IC50 values were determined.*

Results: *Sheep whey proteins were incubated with Caco-2 cells for 24, 48 and 72 hours with serial dilutions starting with 3200 µg/ml and decreasing. When incubated with sheep whey Caco-2 cells, cell viability: 66.34% at 24 h; IC50 value of 8.414 µg/ml; IC50 value of 46.61% at 48 h was 5,838 µg/ml; The IC50 value of 4.61% at 72 h was found to be 4,741 µg/ml.*

Conclusion: *It was concluded that whey proteins obtained from sheep colostrum isolation have apoptotic and anticarcinogenic effects on Caco-2 cancer cell line and significantly inhibit the growth of tumor cells. We think that the results of this study will lead to studies to be carried out in vitro.*

Keywords: Colostrum, colorectal adenocarcinoma, cytotoxicity, whey proteins

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Introduction

Colorectal cancer (CRC) is the third most common cancer type in the world after lung and breast cancer. Every year, approximately 608,000 deaths occur due to CRC in the world[1]. Colorectal cancer usually results from its progressive accumulation with genetic and epigenetic differentiation that transforms normal colon epithelium into colon adenocarcinoma. Chromosomal instability, oncogenic mutation of RAS and BRAF genes, familial predisposition, smoking, age and diet can be counted as causes of colorectal cancer[2]. The composition of colostrum and the substances it contains vary depending on the animal's feeding, environmental and housing conditions, when colostrum is collected from the breast in the postpartum period, and the processing and condensation procedures of colostrum[3]. Along with cystine, colostrum has a rich content in terms of albumin, lactoferrin and lactoalbumin proteins, which plays a role in the transport of iron and copper into the cell[4]. It has been reported that the proteins present in colostrum form a chelate with iron, exert a protective effect in the cell against lipid peroxidation of iron, have a protective effect on the cell in its content, and show anticarcinogen and antioxidant effects due to the conjugated linoleic acid in its content[5]. It is stated that colostrum is an extremely safe natural nutrient, and it has no significant side effects other than mild gastrointestinal complaints such as bloating and nausea in humans[6]. Colostrum consumption by the offspring of ruminant species (cows, sheep, and goats) has a fundamental role in passive immune transfer and neonatal survival[7]. However, it directly affects the immune level of the lamb in the body immune system and plays an important role in protecting the animal from microorganisms[8]. Colostrum quality; It is under the influence of many factors such as the age of the animal, breed, nutritional level before pregnancy, length of stay in the dry period, difficult birth, size and behavioral factors[9].

Materials and Methods

The colostrum sample used in the study was obtained from sheep (Akkaraman) under hygienic conditions. Sheep colostrum was obtained by milking on the 3rd day. The milk was delivered to the laboratory by cold chain. The study was carried out in Dicle University Health Research Center Laboratory. The pH was adjusted to 7.6 if 1 M NaOH was added to the degreased colostrum samples. Then, whey protein was obtained from the supernatant by centrifugation at 4000 g for 30 minutes at 15 °C. Phosphate Buffer Saline (PBS) 3 times the volume taken from the obtained whey proteins was added and centrifuged at 4000g for 10 minutes at 15°C and washed. After washing, whey proteins were purified from microorganisms by passing them through a 0.22 µm membrane filter with the help of Millipore vacuum pump. Colostrum samples, which turned

into powder after 96 hours of lyophilization, were stored at -80ºC until the time of study. In our study, the MTT test, which is one of the cell viability analyzes, was preferred. Whey protein was weighed on a precision balance of 0.032 g and dissolved in 1% PBS (1 ml). After being completely homogenized, it was prepared in sterile eppendorf tubes with serial dilution at different concentrations in a laminar flow safety cabinet. 3.200 µg/ml, 1.600 µg/ml, 800 µg/ml, 400 µg/ml and 200 µg/ml of whey proteins were added to 96-well plates in equal amounts to each well by serial dilution. Then, incubation process was performed for different durations such as 24, 48 and 72 hours. After incubation, 10 µl of MTT solution (5 mg/ml) was added to sterile wells and kept in an incubator containing 5% CO2 at 37 °C for 3 hours. After the incubator, 100 µl of DMSO was added to the wells with the help of a multi-pipette in order to dissolve the formason crystals, and the 96-well plate was covered with aluminum foil and shaken for 10 minutes. At the end of the time, absorbance was measured with a microplate reader (570 nm). The applied protocol was applied within 48 and 72 hours and absorbances were obtained.

The first wells in which only the medium was left were the control group, and the viability of the cells was accepted as 100%. The percent viability rates of the cells were found with the formula given below; % viable cells = Cell absorbance of samples applied at different concentrations / Control cell absorbance × 100

Statistical Analysis

The results of the graphs related to the study were obtained with the Graphpad Prism 8 program (GraphPad Software, http://www.graphpad.com). Statistical analyzes of the MTT study were performed on a computer using SPSS 22 software. ANOVA test was performed to determine the differences between the groups studied in the MTT method. The significance level in different groups was determined according to $p < 0.05$.

Result

The IC50 value, which is the growth inhibiting effect concentration in 50% of the tumor cells, was determined using the excel program. When incubated with sheep whey Caco-2 cells, cell viability: 66.34% at 24 h; IC50 value of 8.414 µg/ml; IC50 value of 46.61% at 48 h was 5,838 µg/ml; The IC50 value of 4.61% at 72 h was found to be 4,741 µg/ml.

Sheep whey Caco-2 cell viability rate

Cell viability rates of sheep whey protein in A549 cells (control 100%) for 24h, respectively, depending on dose and time: 97.34% at 200 µg/ml; 84.89% at 400 µg/ml; 77.0% at 800 µg/ml;

72.45% at 1600 µg/ml; Viability percentages of 66.36% were found at 3200 µg/ml; for 48h: 81.78% at 200 µg/ml; 74.11% at 400 µg/ml; 54.95% at 800 µg/ml; 51.53% at 1600 µg/ml; Viability percentages of 46.61% were found at 3200 µg/ml; for 72h: 81.61% at 200 µg/ml; 80.78% at 400 µg/ml; 69.95% at 800 µg/ml; 38.19% at 1600 µg/ml; Viability percentages of 16.61% were found at 3200 µg/ml.

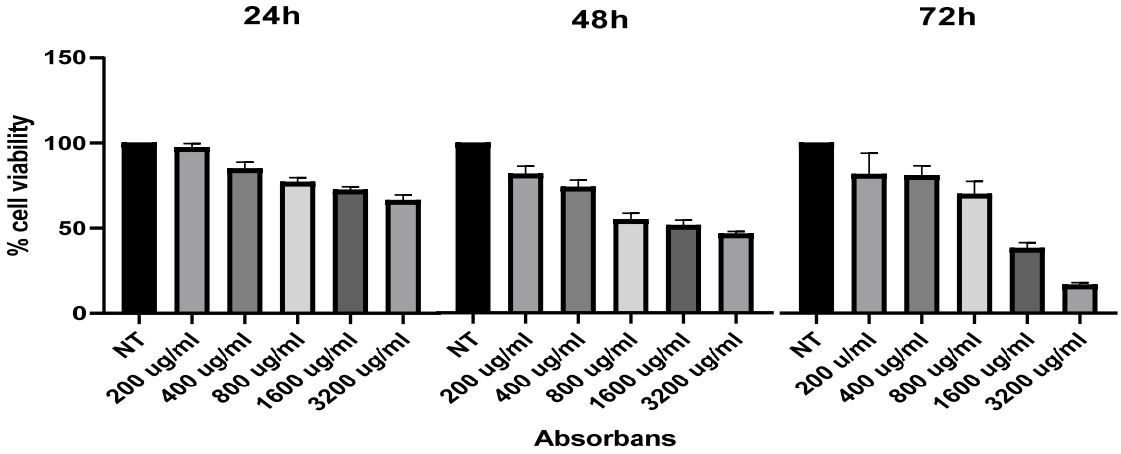

Figure 1. *Cell viability rates determined by MTT in sheep whey Caco-2 cells*

Whey protein in Caco-2 tumor cells in the control group and different concentrations for 24 hours; A significant difference was found between the control group and 400 µg/ml, 800 µg/ml, 1.600 µg/ml and 3.200 µg/ml ((p<0.001); for 48 hours; A significant difference was determined between the control group and other concentrations ((p<0.001); for 72 hours; a significant difference was determined between the control group and the control group and other concentrations ((p<0.001).

Table 1. *Sheep whey Caco-2 findings*

	24h	**48h**	**72h**
	Ort±Sd	**Ort±Sd**	**Ort±Sd**
NT	100±0,00^{ab,1}	100±0,00^{ab,1,2}	100±0,00^{ab,1,2}
200 µg/ml	95,21±2,9^{bb,2}	81,78±4,63^{bb,2}	81,61±12,46^{bb,2}
400 µg/ml	84,89±3,95^{aA,2}	74,11±4,09^{cbA,2,1}	80,78±5,76^{cb,2}
800µg/ml	77,06±2,65^{abB,2}	54,95±3,76^{abB,1,2}	69,95±7,5^{ab,2}
1600µg/ml	72,45±1,81^{ac,2}	51,52±3,10^{acC,1}	38,19±3,19^{bb,1}
3200µg/ml	66,36±3,25^{aC,1}	46,61±1,28^{abD,2}	16,61±1,28^{bbD,2}

a, b,c Absorbances in the same column with different significance levels are expressed with the same letters.

A,B,,C,DA absorbances on the same line are expressed with the same letters.

[1] The statistical difference between the absorbances shown with the same number in the same column is significant (p<0.05). [2] The statistical difference between the absorbances shown with the same number in the same column is significant (p<0.001).

Discussion

Cancer treatment; mainly applied include surgery, chemotherapy, radiotherapy and immunotherapy[10]. In general, chemotherapy, which is frequently preferred in cancer, has a negative effect on healthy cells by showing toxicity in normal cells, which can sometimes cause lifelong irreversible side effects. In addition, although the immune system of the patient receiving chemotherapy decreases, nausea, vomiting, diarrhea, hair loss, fatigue and mouth sores are seen. These disadvantages necessitate the need to develop treatment strategies and treatment supporters with minimal side effects[11]. In our work; We aimed to determine the anticancer role of colostrum components in Caco-2 cancer cells due to the many benefits they provide to human health and to reveal a new horizon in cancer patients.

In the study, lyophilized sheep whey was applied to the colorectal adenocarcinoma Caco-2 cell line for 24, 48 and 72 hours and cell viability rates were determined by MTT analysis.

Proteins in whey; α-lactalbumin, β-lactoglobulin, lysozyme, lactoferrin, serum albumin and immunoglobulins. There are study data that many of these proteins have an anticarcinogenic role[12]. In addition to whey, milk also contains casein protein[13]. In our study, we determined that sheep Caco-2 has a cytotoxic effect at the level of 84% in cancer cells.

Fakharany et al., in their study, showed that albumin protein in human, cow and camel milk inhibited CaCO2, HepG-2, PC-3 and MCF-7 cells against albumin-oleic acid complex dose-dependent tumor cells according to the MTT method[14]. In this study, we found that sheep whey colostrums showed cytotoxic activity against Caco-2 cells, depending on dose and time, according to the MTT method, Fakharany et al. supports its findings. McIntosh et al. concluded in their study that proteins in dairy products, especially whey proteins, play an important role in preventing cancer[15]. In our study, it was revealed that sheep whey proteins killed 84% of cancer cells in 72 hours at a dose of 3,200 µg/ml, which is the highest dose of CaCO 2 in cancer. Karagözlü and Bayerer reported in their study that all of the proteins found in whey show anticarcinogenic effects [16]. In our study, we concluded that whey proteins have an antiproliferative effect against Caco-2 cells.

Conclusion:

In the light of the data we obtained, it was concluded that sheep whey protein has antiproliferative activity on Caco-2 cells. It is thought that our study will contribute to the use of

sheep whey protein in cancer treatment by showing anticancer activity and will shed light on the studies to be done on this subject.

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REVIEW

COVID-19 Associated Autoimmunity: "Are Autoantibodies Neglected?"

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Özet:

Koronavirüsler soğuk algınlığı gibi hafif enfeksiyon tablolarından, daha ağır klinik tablolara neden olabilen büyük bir virüs ailesidir. 31 Aralık 2019'da Çin'in Wuhan şehrinde etiyolojisi bilinmeyen pnömoni vakaları bildirilmiştir. 7 Ocak 2020'de hastalığın adı Coronavirus Disease-2019 (COVID-19) , etkenin adı da SARS-CoV-2 olarak adlandırılmıştır. Araştırmalar, hastalığın kötüleşmesinin immünopatolojik olduğunu göstermiştir. Virüse karşı şiddetli bir immünolojik yanıtın başlaması ve sitokin seviyelerinin yükselmesi sonucu klinik progresyon hızlıca kötüleşmektedir. Şiddetlenen immünolojik yanıtın yanı sıra, bazı çalışmalarda otoantikorların hastalığa etkisi üzerinde durulmuştur. Bazı hastalarda kendi hücre ve dokularını hedef alan otoantikorlar rapor edilmiştir. Bu otoantikorların nasıl oluştuğu tam olarak bilinmese de, immün sistemin kişinin kendi hücrelerine karşı duyarlılaşması ve virüse ait epitoplardan bazılarının kendi antijenlerimizle benzerlik gösterebileceği teorileri üzerinde durulmaktadır. Otoantikorların hastalığın şiddetini arttırarak iyileşme sürecini uzattığı gösterilmiştir. COVID-19 vakalarında en sık (Anti-nükleer antikor) ANA, anti-fosfolipid antikorları ve anti-tip 1 interferon antikorları saptanmıştır. Nadiren diğer otoantikortürlerine de (Anti-nötrofil sitoplazmik antikor (ANCA), Anti-siklik sitrullin peptid antikor (Anti-CCP) vb.) rastlanmıştır. COVID-19 hastalığında otoantikorların oluşumu ile ilgili ileriye yönelik daha çok sayıda bilimsel araştırma yapılması gerekmektedir.

Anahtar kelimeler : *COVID-19, SARS-CoV-2, Otoantikorlar, Anti-Nükleer Antikorlar, Anti-Nötrofil Sitoplzmik Antikorlar*

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Abstract:

Coronaviruses are a large family of viruses that can cause mild infections, such as the common cold, to more severe clinical manifestations. On 31 December 2019, cases of pneumonia of unknown etiology were reported in Wuhan, China. On 7 January 2020, the name of the disease was named Coronavirus Disease-2019 (COVID-19), and the agent was named SARS-CoV-2. Studies have shown that the worsening of the disease was immunopathological. Clinical progression rapidly worsens as a result of the onset of a severe immunological response to the virus and the elevation of cytokine levels. In addition to the intensified immunological response, some studies have focused on the effect of autoantibodies on the disease. Autoantibodies targeting their own cells and tissues have been reported in some patients. Although it is not known exactly how these autoantibodies are formed, theories are focused on the sensitization of the immune system to one's own cells and that some of the epitopes of the virus may resemble our antigens. Autoantibodies have been shown to increase the severity of the disease and prolong the healing process. (Anti-nücleer antibody) ANA, anti-phospholipid antibodies and anti-type 1 interferon antibodies were detected most frequently in COVID-19 cases. Rarely, other types of autoantibodies -Anti-neutrophil cytoplasmic antibody (ANCA), Anti-cyclic citrulline peptide antibody (Anti-CCP) etc.- have been encountered. More comprehensive prospective scientific studies should be conducted on the formation of autoantibodies in COVID-19 disease.

Keywords : COVID-19, SARS-CoV-2, Autoantibodies, Anti-Nuclear antibodies, Anti-Neutrophil Cytoplasmic Antibodies

Introduction

Coronaviruses (CoV) are a collection of viruses that can cause mild infections, usually in the form of the common cold. SARS-CoV (Severe Acute Respiratory Syndrome Coronavirus) first detected in humans in 2002 and MERS-CoV (Middle East Respiratory Syndrome Coronavirus) in 2012 caused fatal epidemics [1,3,6]. The third coronavirus epidemic in the world started with the notification of pneumonia cases of unknown cause in Wuhan, China by the World Health Organization (WHO) in December 2019. Later, the name of the disease was accepted as Coronavirus Disease-2019 (COVID-19) and the agent was named SARS-CoV-2. The first COVID-19 case in our country was seen on March 11, 2020 [1]. The World Health Organization declared the epidemic as a pandemic on March 11, 2020 [2].

Virological Characteristics of SARS-CoV-2

Coronaviruses are enveloped single-stranded RNA viruses in the Coronaviridae family. These viruses are named Coronavirus due to the presence of protrusions on their surfaces called “corona”, meaning “crown” in Latin [3,4]. SARS-CoV-2 is in the Betacoronavirus genus. The genetic structure of this genus was shown to be similar to the genome of the bat-derived coronavirus [5].

Pathogenesis of COVID-19: "What's going on in the immune system?"

The pathogenesis of COVID-19 can be examined under three headings: virus proliferation, overstimulation of the immune system and multi-organ failure [7]. First, the virus replicates in host cells and infects new cells, causing damage to the lung parenchyma. In addition, acute respiratory distress syndrome (ARDS), sepsis, and multi-organ failure develop as a result of excessive cytokine production by over-stimulating the immune system [8,9]. Some studies show that a severe immune response is more important than a direct virus-specific lethal effect in COVID-19 [10]. Thus, with the progression of the disease, the overstimulation of the immune system and the serious increase in cytokine levels indicate that serious organ damage may be of immunopathological origin [11,12].

COVID-19 Immunopathogenesis

Studies have shown that the Coronavirus Spike protein is an important structural fragment for the virus to enter target cells [13]. This molecule enters the cell by binding to the Angiotensin converting enzyme-2 (ACE-2) receptor on the target cell surface [14,15]. Then, the virus RNA is released into the cytoplasm and all the structures belonging to the virus are synthesized. The resulting viruses are released out of the cell by budding [14,16-18]. Lung epithelial cells begin to secrete interleukin-8 (IL-8), which has a stimulating effect on neutrophils and T lymphocytes [19]. The innate immune response is initially induced by lung epithelial cells, alveolar macrophages, and neutrophils. Afterwards, T and B lymphocytes take part[20].

RNA-containing viruses are recognized by macrophages and initiate the innate immune response. This causes the synthesis of IL-1, IL-6 and Type 1 interferon (IFN1) [21]. In addition, neutrophils work towards the infection site to destroy the virus [22]. As a result of antigen presentation, abundant cytokines begin to be synthesized. Antigen-specific cytotoxic T lymphocytes destroy infected cells, while B cells produce virus-specific antibodies [23,24]. As in other viral infections, virus-specific IgM and IgG-type antibodies are formed. IgG antibodies that are specific to the virus are of the protective type [25]. It has been shown that IgG and IgM levels are higher in severe cases than in mild cases [26]. In addition to these specific antibodies in COVID-19 patients, the formation of autoantibodies, which can increase the severity of the disease by attacking some cells and tissues, has also been a matter of curiosity.

What is Autoantibody? Why does it occur?

The most important feature of a normal immune system is that while immune system cells respond to many foreign antigens, they do not respond to their own antigens. Thanks to this discrimination ability, while immune system cells attack microorganisms, no defense response occurs against the body structures. Our immune system acquires this feature, called immune tolerance, in the bone marrow and thymus while still in the mother's womb. Meanwhile, T and B lymphocytes that respond to the body's self-antigen are eliminated by various mechanisms [27]. This tolerance mechanism is called central tolerance. Another mechanism is the peripheral tolerance mechanism [28]. Central tolerance is achieved in two ways. First, immune-reacting cells are destroyed by apoptosis when self-antigen is presented to them. The second is the expression or anergy (reducing receptor expression) of a new Fab receptor that does not respond when bound to self-antigen. Thus, autoreactive cells are destroyed during the construction phase. However, if the central tolerance mechanism cannot function completely, some T and B lymphocytes may manage to escape this mechanism [29]. In this case, escaping autoreactive lymphocytes are destroyed by the other mechanism, peripheral tolerance. Peripheral tolerance occurs in lymph nodes and tissues. Here, autoreactive lymphocytes are inactivated by mechanisms such as apoptosis and anergy. Peripheral tolerance functions in this way and acts as a second defense mechanism [30].

Thanks to tolerance mechanisms, although a healthy individual tries to protect own antigens from own immune system, unresponsiveness to own antigens may disappear in some cases. Some of the immune system cells do not recognize their own tissue antigens and begin to perceive them as a foreign antigen, and these cells attack the individual's own cells and tissues [31]. This condition is called **autoimmunity**, and antibodies against self antigen are called **autoantibodies**. Autoantibodies can damage various structures in the body, either intracellular or extracellular. For example, antinuclear antibodies (ANA) to structures such as DNA, RNA, nucleolus in the cell nuclei of all systems in the body, anti-phospholipid antibodies to the circulatory system by affecting the phospholipids in the cell membrane, Anti-neutrophil cytoplasmic antibodies (ANCA) to neutrophils and therefore to the vessels, parietal cell antibodies to the stomach, thyroglobulin antibody to the thyroid gland, acetylcholine receptor antibody damages the muscles [32,33]. It has also been reported that autoantibodies can be formed against cytokines [34].

Although it is not known exactly why autoimmunity is triggered, some possibilities are being considered. One of these is the introduction of an antigen that cross-reacts into the organism. Antibodies produced against these antigens are thought to respond to self-antigens after a while

[35,36]. The second possibility is that some drugs and chemicals affect immune system cells and some chronic infections cause changes in the body's own antigens. These structural changes in self-antigens cause the immune system cells to give autoreactive responses [37]. A third possibility is failure of immune tolerance due to genetic causes (especially Major Histocompatibility Complex (MHC) genes). When this process is insufficient, autoantibody formation is triggered [38]. In addition, T lymphocyte dysfunction may also cause autoantibody formation. In this case, T lymphocytes cause B lymphocytes to produce antibodies against self-antigens that they see as foreign [39].

COVID-19 Associated Autoantibodies

In the COVID-19 disease caused by the SARS-CoV-2 virus, which was identified at the end of 2019, the immune system interferes with this situation when the virus enters the body. Virus-specific antibodies are rapidly produced. It is known that first IgM levels increase and then IgG antibodies increase. These antibodies help to heal by playing an important role in the fight against the virus [40]. However, in some patients, apart from the antibodies produced specifically for the virus, autoantibodies targeting their own cells and tissues were also found [41]. Although it is not known exactly how these autoantibodies are formed, two possibilities are considered. The first is that in repeated coronavirus infections, the immune system becomes so sensitized that it damages one's own cells. Second, some of the epitopes of the virus are similar to our own antigens. It is thought that some of the antibodies formed against the virus in this way also damage our cells [35]. It has been observed that the autoantibodies formed are one of the important factors affecting the severity of the disease. Autoantibodies have been held responsible for severe symptoms that may even lead to death in some patients [42]. It has been reported that ANA, antiphospholipid antibodies and anti-type 1 interferon antibodies are most frequently encountered in COVID-19 cases, as well as other types of autoantibodies rarely [35].

Anti-Nuclear Antibody (ANA)

ANA is a common autoantibody in autoimmune diseases such as Systemic Lupus, Sjögren's and Systemic Sclerosis. These autoantibodies attack structures such as DNA, histones, and centromeres in the cell nucleus, causing widespread tissue damage and inflammation. It is the most common type of autoantibodies in COVID-19 patients. ANA was reported positive in approximately **40-50%** of the patients [43,44]. In addition, a relationship was stated between disease severity and ANA level [45]. While the ANA level was found to be high in those with severe disease, lower levels of ANA were detected in those with mild disease. Although approximately 10% of healthy people may also have ANA, this high rate indicates that COVID-19 triggers autoimmunity.

Antiphospholipid Antibodies

Phospholipids are compounds found in the membranes of all cells, including blood cells and endothelial cells that line the vessel wall. Antiphospholipid antibodies target these compounds. It is thought that these antibodies, which attach to the phospholipids in the cell membrane, initiate coagulation [46]. These autoantibodies have been blamed for disseminated intravascular coagulation in critically ill COVID-19 patients, and thrombosis has had serious consequences that can lead to death [47].

Anti Type 1 Interferon Antibodies

Type 1 interferons are antiviral cytokines that function in the immune system. They are synthesized by fibroblasts and monocytes to prevent virus attack. Type 1 interferons bind to specific receptors on target cells, resulting in the formation of proteins that prevent the replication of viruses within the cell. Thus, the proliferation of the virus in the cell is prevented and the spread of the virus to other tissues is prevented [48]. In particular, autoantibodies against type 1 interferons were found in approximately 10% of patients with severe COVID-19 [42]. It is thought that these autoantibodies bind to type 1 interferons, preventing their function and accelerating the spread of the virus. Therefore, it was concluded that one of the reasons for having a severe disease may be due to the presence of anti-type 1 interferon antibodies. It has also been observed that these autoantibodies affect on the disease in healthy young people [49].

Conclusıon

Autoantibodies have been found at a lower rate in COVID-19 patients than in other diseases. These autoantibodies have been shown to increase the severity of the disease and prolong the healing process. On the other hand, it has been found that a large amount of autoantibodies formed during the disease period can remain in the body for a long time after the disease [41]. This shows us that the effects of autoantibodies can continue even after the disease has healed. In rare cases, it has been found that autoantibodies may form after a certain period of time after the disease has healed [50]. For this reason, even if the pandemic ends, it would not be correct to say that the effects of the disease will disappear completely.

In recent years, case reports have been reported that SARS-CoV-2 vaccines also cause autoantibody formation [51]. The fact that approximately 85% of the population in our country is vaccinated makes the data of these studies valuable. Although vaccination has the risk of developing autoantibodies, a large community immunity has been created in the world with this practice, the number of intensive care patients and the stress caused by the pandemic on nations have decreased. For this reason, we think that the necessity of vaccinations is not open to

discussion. However, we believe that further scientific research is needed to determine whether SARS-CoV-2 and vaccinations cause autoantibody formation.

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