J Immunology Clinical Microbiology
ISSN (online): 2528-9470

Journal of Immunology and Clinical Microbiology

2024;
Volume 9, Issue 3

Citation Abbreviation:
J Immunol Clin Microbiol

Medicine & Publishing

Published by QMEL®.org
(Quality in Medicine,
Education & Library)

www.Jtacm.com

JOURNAL OF IMMUNOLOGY AND CLINICAL MICROBIOLOGY

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Journal Of Immunology And Clinical Microbiology

Cilt/Volume:9, **Sayı/Issue:**3, 2024

Sahibi/Owner: QMEL adına Erkan YULA'dır .

Yayınlayan/Publisher: Erkan YULA

E-Posta/E-mail:erkanyula@gmail.com

Yayın Tarihi/Release Date: 30 Eylül 2024

e-ISSN: 2528-9470

Journal Of Immunology And Clinical Microbiology yılda 4 kez yayınlanır.
Derginin yayın dili Türkçe ve İngilizce'dir.
Makale gönderim adresi: https://dergipark.org.tr/tr/pub/jicm

Yayımcı/Publisher: Cetus Publishing
İletişim/Contact:+90 850 380 08 02
Eposta/Email:info@cetuspub.com
İnternet Adresi/Website :ww.cetuspub.com

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Journal of Immunology and Clinical Microbiology
İmmünoloji ve Klinik Mikrobiyoloji Dergisi
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DOI: 10.58854/jicm.1465143

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ORIGINAL ARTICLE / ÖZGÜN MAKALE

A Framework to Connect Viral Quasispecies, Microbiome, and Host

Mikrobiyom ve Konak Arasında Bağlantı Kurmak İçin Bir Çerçeve

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Geliş: 04.04.2024, Kabul: 30.09.2024

Abstract

Aim: The aim of this study is to investigate the potential interactions between SARS-CoV-2 Spike protein variants and the host microbiota. While the Spike protein is known for its role in mediating viral entry into host cells, its impact on the host's microbial communities remains unclear. Given the microbiota's critical role in modulating immune responses and maintaining host homeostasis, understanding these interactions could provide new insights into disease progression and immune evasion mechanisms associated with COVID-19. By leveraging parameters extracted from the current literature and analyzing publicly available datasets, we seek to elucidate how these interactions might influence the severity of COVID-19 and the pathogenesis of emerging viral variants. This research may also highlight potential therapeutic targets for mitigating the effects of SARS-CoV-2 and its evolving forms.
Methods: This study investigates the interaction between Spike protein variants of SARS-CoV-2 and the host microbiota. To this end, the associations between various SARS-CoV-2 variants and different host factors derived from urban ecosystems have been statistically analyzed. Specifically, the influence of these host factors, which are linked to distinct microbiota compositions, on the interaction with Spike protein variants has been evaluated. A Bayesian Network approach has been employed for this analysis to model the complex relationships and dependencies among the host factors and microbiota compositions.
Results: This study investigates the interaction between Spike protein variants of SARS-CoV-2 and host factors. Hypothesis 1 (H1) posits that specific combinations of various host factors can explain the infectivity of SARS-CoV-2. The analyses reveal that 20 SARS-CoV-2 variants and mutants are significantly affected by various parameters (Table 2), indicating that H1 cannot be rejected. Additionally, it is suggested that the connections mentioned in H1 indicate the presence of a carrier within the host, potentially the microbiome. Hypothesis 2 (H2) proposes that the microbiota serves as the primary carrier of host factors, influencing the selection of specific SARS-CoV-2 mutants. To test this hypothesis, a Bayesian Network was constructed (Figure 3), which identified the probabilistic relationships between potential microbiota compositions and Spike variants.
Conclusion: As a result, it is suggested that different Spike protein variants may be present in hosts with varying microbial compositions. Additionally, the microbiota could serve as a carrier that influences the selection of viral mutants in hosts within the population, potentially impacted by external factors such as environmental conditions and human interactions.
Keywords: COVID-19, Microbiome, Spike Protein, Viral Variant, Host Factor

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How to Cite: Nehri LN, Koçoğlu SE. A Framework to Connect Viral Quasispecies, Microbiome, and Host. *Journal of Immunology and Clinical Microbiology* 2024;9(2):73-88

Öz

Amaç: Bu çalışmanın amacı, SARS-CoV-2 Spike proteini varyantları ile konak mikrobiotası arasındaki olası etkileşimleri araştırmaktır. Spike proteininin virüsün konak hücrelere girişini sağlamadaki rolü iyi bilinmesine rağmen, bu proteinin konak mikrobiyal topluluklar üzerindeki etkisi belirsizliğini korumaktadır. Mikrobiotanın bağışıklık yanıtlarını düzenlemede ve konak homeostazını sağlamadaki kritik rolü göz önüne alındığında, bu etkileşimlerin incelenmesi, COVID-19'un hastalık ilerleyişi ve bağışıklık kaçışı mekanizmaları hakkında yeni bilgiler sağlayabilir. Literatürdeki mevcut parametreler ve halka açık veri setleri kullanılarak bu etkileşimlerin COVID-19'un şiddeti ve ortaya çıkan virüs varyantlarının patogenezi üzerindeki etkileri araştırılmıştır. Bu araştırma aynı zamanda SARS-CoV-2 ve gelişen varyantlarının etkilerini hafifletmek için potansiyel terapötik hedef olarak mikrobiyotayı ortaya koymayı hedefler.

Yöntem: Bu çalışmada, SARS-CoV-2'nin Spike protein varyantları ile konak mikrobiota arasındaki etkileşim incelenmiştir. Bu amaçla, çeşitli SARS-CoV-2 varyantlarının kentsel ekosistemlerden elde edilen farklı konak faktörleriyle ilişkileri istatistiksel olarak analiz edilmiştir. Özellikle, bu konak faktörlerinin, farklı mikrobiota kompozisyonları ile olan etkileşimleri değerlendirilmiştir. Analiz için, konak faktörleri ile mikrobiota kompozisyonları arasındaki karmaşık ilişkileri ve bağımlılıkları modellemek amacıyla Bayesian Ağı yaklaşımı kullanılmıştır.

Bulgular: Bu çalışmada, SARS-CoV-2'nin Spike protein varyantları ile konak faktörleri arasındaki etkileşim incelenmiştir. Hipotez 1 (H1), çeşitli konak faktörlerinin belirli kombinasyonlarının SARS-CoV-2'nin enfektifliğini açıklayabileceğini öne sürmüştür. Analizler, 20 SARS-CoV-2 varyantı ve mutantının çeşitli parametrelerden önemli ölçüde etkilendiğini göstermiştir (Tablo 2). Bu sonuç, H1'in reddedilemeyeceğini ortaya koymaktadır. Ek olarak, H1'de belirtilen bağlantıların, konak içinde bir taşıyıcı olduğuna ve bunun mikrobiom olabileceğine işaret ettiği düşünülmektedir. Hipotez 2 (H2) ise, mikrobiotanın konak faktörlerini taşıyarak belirli SARS-CoV-2 mutantlarının seçimini etkileyen ana yapı olduğunu önermektedir. Bu hipotezi test etmek amacıyla oluşturulan Bayesian Ağı (Şekil 3) ile olası mikrobiota kompozisyonlarının Spike varyantları ile olasılıksal ilişkisi tespit edilmiştir.

Sonuç: Sonuç olarak, farklı Spike protein varyantlarının farklı mikrobiyal kompozisyonlara sahip konaklarda bulunabileceği önerilmektedir. Ayrıca, mikrobiota, konaklardaki viral mutantların seçimini etkileyebilecek bir taşıyıcı rolü üstlenebilir; bu etki, çevresel koşullar ve insan etkileşimleri gibi dış faktörlerden etkilenebilir.

Anahtar Kelimeler: COVID-19, Mikrobiyom, Spike Proteini, Viral Varyant, Konak Faktörler

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), which is shared by many organisms such as bats, pigs, cats, and humans (1). The SARS-CoV-2 virus belongs to the SARS-MERS viral family in the evolutionary pathway, and variants of these diseases have been seen before (2). SARS-CoV-2 is an RNA virus that belongs to the Nidovirales order and Coronaviridae family (3). SARS-CoV-2 is evolutionarily related to HCV-229E, NL63, OC43, and HKU1, which are viruses that cause common colds in 15-30% of humans (4). Viruses belonging to the Nidovirales order exhibit similar structural features (3). Nidoviruses have few structural proteins and RNA as their genetic material, along with a lipid envelope that protects the genetic material from the environment (3). All Nidoviruses contain a Nucleocapsid (N) protein that interacts with the Membrane protein (M); however, both structures and proteins vary among the viruses (3; 5). The genome sizes vary among the Nidoviruses, while the genome structures remain similar. All genomes possess two large Open Reading Frames (ORFs) that hold the genetic information of proteins responsible for transcription regulation. The parts for structural proteins (such as M and N) are located in the genome near the ORFs (3; 5). The life cycle of SARS-CoV-2 consists of four stages: the attachment of the virus to the cell and transfer of genetic material, processing of genetic material, assembly of viral proteins resulting from translation, and release of the unified virions from the cell (6). The interaction of viral proteins

with various host proteins has also been the subject of many studies (6; 5). SARS-CoV-2 proteins associate with certain host proteins, forming complexes that alter the virus's effect on the host (2). For instance, a virus-host protein-protein interaction (PPI) formed by TOM-70 (a host cell membrane protein) and Orf-9b (a SARS-CoV-2 protein) exemplifies this type of relationship. Such SARS-CoV-2 virus-host protein interaction pathways can also be associated with MERS and SARS-CoV viruses, making them potential targets for drug development due to their shared patterns.

The SARS-CoV-2 genome consists of two ORF parts that encode non-structural proteins. In addition to the two ORFs, four structural gene regions carry the genetic information for the virus's structural proteins (6). In SARS-CoV-2, 16 nonstructural proteins (derived from the cleavage of the two large ORF proteins), four structural proteins (spike (S), envelope (E), membrane (M), and nucleocapsid (N)), and eight accessory proteins are present (5). The polyproteins of Orf1a and Orf1b are cleaved into smaller non-structural proteins (NSPs). NSPs interact with each other to regulate gene expression, while the Membrane protein forms the virus's lipid membrane. The Nucleocapsid protein links to the Membrane protein and encapsidates the RNA genome. The Envelope protein is an integral membrane protein that creates an ion channel and plays a role in the virus replication process. The Spike protein is the surface glycoprotein that mediates the attachment of host cells to the virus (5).

Spike protein is one of the most important structural proteins of SARS-CoV-2 (7). This protein recognizes and binds to the human host cell surface receptor angiotensin-converting enzyme-2 (ACE2), providing entry into the cell. The host's immune response is also triggered by the detection of the Spike protein (7). Moreover, the Spike protein determines the infectivity and transmissibility of the virus and is the major antigen inducer for the immune response (6). Therefore, many vaccines have been designed to target the Spike protein (5). The Spike protein consists of two subunits: S1 and S2. S1 is responsible for binding to ACE2 receptors, and after this binding process, the S2 subunit facilitates fusion into the cell, allowing the virus's genetic material to enter (6). The cleavage of the S1 subunit from S2 is critical for infection; therefore, antibodies bind to the Spike protein to prevent cleavage and inhibit the virus's fusion with the cell (6).

SARS-CoV-2 exists as a haplotype in its host as an RNA virus, and Spike proteins can also be categorized through haplotype analysis (8). Haplotypes represent cumulative variations in genetic data on a single chromosome (9). In haplotype variations, a variant is dominant among the others, with these variants occurring at very low frequencies compared to the dominant haplotype (9). Clusters of mutants surround this main haplotype, with sequence similarities ranging between 93% and 99% among the dominant haplotype (9). In other words, the haplotype distribution in a host displays a scenario where one haplotype is central to the viral population, with some mutants present around it. Computational experiments have been conducted to verify these facts using experimental data and specific software (9; 5). These findings are applicable to RNA viruses as well. Since RNA viruses exhibit low recombination levels and lack true diversity in the conserved regions of their genome, haplotype distributions are minimal, and mutations accumulate around one or two main haplotypes (9; 5). Consequently, these closely-related haplotypes in viral populations form viral quasispecies—defined as the dynamic distribution of closely related but non-identical mutant and recombinant viral

genomes—or, in other words, quasispecies represent viral groups within a population composed of haplotype variations (9). These quasispecies function as a unit of selection due to their variation (9; 5).

There are two main causes of variation in a viral population: recombinations and mutations (10-12). Even though mutations and recombination events are high in non-conserved regions, they are rare in conserved regions. In viral quasispecies, there is a dominant haplotype that shows very low recombination events in its evolutionary history, with many mutant types surrounding these major dominant haplotypes (13). Specifically, they tend to identify haplotype probabilities—which represent the architecture of the viral population in a host—that shape host interactions. Spike protein is not an exception; the protein, along with SARS-CoV-2, is found in the host as a haplotype structure (14). Spike proteins are made up of small differences between different haplotypes that evolved from the same ancestor (12). The Receptor Binding Domain (RBD) of the Spike protein, which binds to human ACE2 receptors, is not a recent acquisition by recombination but rather an ancient gain that is common to bat viruses (15). Therefore, mutations (such as deletions and insertions), rather than recombination, have great importance in Spike protein (and SARS-CoV-2) evolution, generating Spike protein variants (15). As its evolution rate is similar across clades of SARS-CoV-2 variants, Spike protein is the major evolutionary driver, and SARS-CoV-2 variants are largely categorized according to Spike protein variants (12). In summary, the distribution of SARS-CoV-2 variants in the host aligns with our general understanding of viral haplotype and quasispecies structure.

Microbiomes, which can be defined as the assemblage of microbes in a host, are representatives of the diseases or health condition of the host (16). Microbiomes are key indicators of singular attributes directly related to the host (17), and genetic problems of the host can be detected from its microbiome content (18). For instance, the effects of endocrine-disrupting chemicals (EDCs) in the air can be observed in the lung microbiota of terrestrial animals (19), and the gut microbiota is another target for EDCs (20). Since human microbiomes are major representatives of the host's attributes—such as diet, lifestyle, and medical record—as a whole (21), changes in microbiome content can infer the evolutionary forces acting on the host (17). In microbiomes, ecological relations among species exist. The dominant species, also called founder species, alter the host's biological reactions by providing certain chemicals (22). For instance, the presence of a species can alter the host's immune response by triggering the production of more IgA, which affects the immune response, especially in respiratory areas, as the first line of immune defense (23). The dominant species and other species change between health and disease conditions within a microbiome (24). There are many different characteristics of species within a microbiome. For example, dominant species are often in a positive relationship with other members of the microbiome, usually creating a mutualistic environment, while keystone species have a high number of both positive and negative relationships with other microbes. Keystone species are often found in low abundance but have a high number of ecological connections with other species in the microbiome (22). Moreover, it is known that the abundance of species in the intestinal microbiota is related to the diseases and clinical blood markers of the host organism (25). The microbial composition—viruses, fungi, and bacteria—in the microbiota contributes to many metabolic functions of the host and plays a role in many physiological processes,

especially the immune response (26). The term dysbiosis is used to describe situations where changes in the microbiota are directly related to a host's illness (24). This term indicates that a microbiota community is directly related to a disease in the host, and when the host does not have this disease, the composition of the microbiota is significantly different from the disease state (24). To sum up, the microbiome is an area that has been studied under various conditions. The composition of the microbiota and the relative abundances of the organisms within it are related to both the disease and health conditions of the host (27).

Meta-community is a set of local communities that are linked by the dispersal of multiple potentially interacting species (28), and a microbial meta-community is a variational set of local (e.g., in some host organisms or a geographic area) microbe communities (29). Microbiomes are key indicators of certain attributes that are directly related to the host. For instance, genetic problems can be detected from microbiome content, or the host's lifestyle can be influenced by its microbiome. Human microbiota compositions show discontinuous rather than continuous variation of microbes; in other words, the microbes in the gut form certain clusters (30). These distinct microbial sets are called enterotypes, and three types of enterotypes—with different dominant species and different microbial compositions—have been identified in human microbiota (30). Enterotypes indicate a balanced relationship between the host and its microbiota (30; 31; 19). The most important characteristic of microbiota composition is the functional relationship among microbes, rather than which specific bacterium is present (30). Microbiota shows phylogenetic variation at the genus and phylum levels among enterotypes and functional variation at the class level (30; 32; 33). For instance, the Firmicutes and Bacteroides phyla are the most dominant species in the gut microbiota (34). Although Bacteroides generally dominate the gut microbiome, in some enterotypes, Firmicutes can be the dominant organism (22; 30; 35). Actinobacteria, the most common phylum after Firmicutes and Bacteroidetes, is considered a keystone taxon in the gut microbiota due to its extensive ecological network with other gut microbes (22). Proteobacteria, another common species in the human intestinal microbiome, represents functional variation that occurs in the gut microbiome among different microbial compositions (36). Microbes in the gut microbiota are exposed to selective forces from both host factors, such as diet and disease, and from other microbes in the gut (37). This explains why some low-abundance bacteria survive in the gut (30). Every bacterium in the gut follows a different survival strategy, and typically, the most abundant function is associated with the most dominant type (24; 38). However, since no single dominant species can provide all functions, the functional composition of different species is crucial for the microbiota (30; 35; 39). The composition of the human microbiome is influenced by many factors. For instance, human intestinal microbiota varies geographically (35), influenced by factors such as genetics, lifestyle, climate, diet, and altitude (40). Nevertheless, despite the numerous factors affecting the microbiome, enterotype variations are believed to be independent of age, gender, BMI, and geography, though they are closely related to dietary habits (30; 35). Furthermore, the mucosal immune system, which plays a crucial role in immunity, can be affected by various factors. It is believed that this system can become dysregulated due to intestinal issues. Studies have also shown that the overall immune response is shaped by cross-talk between the gut and the lungs at the organismal level (41).

Several studies highlight the relevance of gut-lung microbiota cross-talk to COVID-19 (42). The microbiome is both affected by diseases and influences disease conditions. For example, microbiota has been linked to diseases such as rheumatoid arthritis, type 1 diabetes, inflammatory bowel disease (IBD), allergic diseases, systemic lupus erythematosus (SLE), skin-related autoimmune pathologies, neurological inflammatory diseases, and various types of cancer (43). The composition of the microbiota also changes during COVID-19 (44). Viral infections in the respiratory tract and lungs can alter the gut microbiota by affecting its function and composition (45), as the intestinal microbiota is associated with the lung microbiota, and changes in the lung microbiome can affect other microbial compositions (46). Moreover, microbiota prevents pathogen invasion through various strategies, such as directly killing pathogens, supporting the host's immune system, or competing for resources (47).

Figure 1. A network representation of some linkages in the literature where Sars-CoV-2 variants and host factors are connected.

In this study, we investigated the association of Sars-CoV-2 variants with both host factors and bacteria in the gut microbiota. For this, both classical statistical analyses and Bayesian Network, a probabilistic approach, were used.

METHODS

The study has 2 hypotheses:

H1: Specific combinations of various host factors can explain SARS-CoV-2 infectivity between variants and specific mutations on Spike protein.

H2: Microbiota is the main carrier of host factors inside the body which specific SARS-CoV-2 mutant is selected by the host.

From the data obtained through the literature research for this study, it has been determined that many different parameters in human life are related to both microbiota and COVID-19 (see Figure 1). To investigate these relations, a generative theoretical explanation was needed. As a result of the literature reviews, the relationship of microbiota with many parameters has been confirmed (see Table 1, Figure 1). Additionally, literature findings indicate that various parameters are associated with COVID-19, and it is known that COVID-19 causes changes in the host's microbiota composition (see Table 1, Figure 1). However, it is essential to investigate how different variants and Spike protein mutations are tolerated by various microbiota. There is no other virus like SARS-CoV-2 that provides detailed global data and the global distribution of its different mutants. Globally, the closest available data belongs to the SARS-MERS family, but even that does not come close to the breadth of data available for SARS-CoV-2 (48). For this reason, the results of the study could not be tested with a second virus as a control group.

Table 1. Independent variables and their relations with COVID-19 and Microbiome via some examples from the literature

Independent variable:	Relatedness with COVID-19 and/ or Microbiome:
Population size (in number)	[84], [100]
Urbanization percentage of the population	[100]–[103]
Deaths by indoor air pollution rates	[104]
Deaths by outdoor air pollution rates	[104]–[106]
Deaths by Covid-19 (in number)	[107], [108]
GDP per capita	[78], [109]
Gini index (income inequality)	[79]
Conflict cases	[79], [109]
Corporate Tax Rates	-
Average Household Size: Number of members	[110], [111]
Prevalence of Total Overweight Adults	[112], [113]
Consumption of the Vegetable Oil	[114], [115]
Consumption of the Animal Fat	[115]–[117]
Consumption of Sugars	[118], [119]
Prevalence of undernourishment by percentage	[120], [121]
Prevalence of Vitamin A deficiency	[122]–[124]
Vitamin D status Around the World	[125], [126]
The global prevalence of Zinc Deficiency	[127]–[129]
Iodine Levels	[69], [130]–[132]
Exposure to Solar UV Radiation	[80], [133], [134]
Average temperature	[82], [135]
Forest Area	[136], [137]
Average Precipitation	[138], [139]
Air Toxicity Levels	[81], [140]
General Toxicity Levels	[141], [142]
CO2 Emissions per capita	[143], [144]
Anemia in pregnant women	[145], [146]
CANCER (For All Types of Cancer)	[147], [148]
Lung Cancer	[149], [150]
Asthma	[151], [152]
COPD	[153], [154]
Pneumonia	[155], [156]
NDCs (Non-communicable Diseases)	[157], [158]
Diabetes	[159], [160]
Diarrheal Diseases	[161], [162]
Colorectal Cancer	[163], [164]

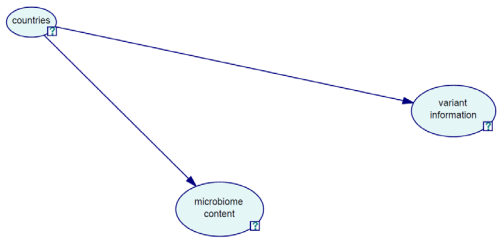

Figure 2. The Bayesian Belief Network for three nodes of countries, variant information, and microbiome content (Created by GeNIe 4.0 Academic).

The CoVariants section of the GISAID database was used to obtain data on city populations that are related to different mutants of Spike protein and variants of SARS-CoV-2. In this section, 58 countries were found with related information. 56 of 58 countries have the relevant variant and mutant data were 32 selected for further analysis (Supplementary Material: 'Data_ mutants' & 'Data_parameters').

A data file containing the data of all members of the parameter sets for the selected countries and the country names was created as a table analysis (Supplementary Material: 'Data_parameters'). Each data column includes data from a single data source -only one web page or database- to provide consistency among data sets for the countries (Supplementary Material: 'Data Sources_Variable Information'). If the data is unavailable in these sources the entry about this data was settled as NULL. These variables were used as independent variables for the analysis. The CoVariants / Per Variant section of the GISAID database was used to obtain mutant and variant data of countries. A data file containing the data of all mutants and variants on the GISAID database for the selected countries' analysis (Supplementary Material: 'Data_mutants'). The maximum frequency of mutants and variants for each country was used for the analysis as dependent variables. Stepwise regression analyses including all independent variables were performed to get the regression

equations to describe the variance between frequencies of the variants of the virus.

Using the significant results from this analysis (see Table 2 and Supplementary Material: 'Data_mutants'), the relationship between gut microbiota and SARS-CoV-2 mutants was represented by a Bayesian Network. As microbiota data, the bacterial distribution of gut microbiota from Mobeen's (2018) study was used for seven countries (Indonesia, India, Japan, Sweden, USA, Italy, and Spain) (35). These countries provided the frequency distribution of four types of bacteria in the gut microbiome—Bacteroides, Firmicutes, Actinobacteria, and Proteobacteria—which are common among various host microbiomes with functional effects. This distribution was used as prior probabilities in the Bayesian Belief Network, as Bayesian approaches are beneficial when data is limited, allowing the incorporation of prior knowledge (Bland & Altman, 1998). To connect the mutant data and microbiome data, the Bayesian approach was employed since the dataset is limited to the distribution of microbiomes across only seven countries. To construct the Bayesian Network, GeNIe 4.0 Academic was used (see Figure 2).

RESULTS

Hypothesis 1 (H1)*: Specific combinations of various host factors can explain SARS-CoV-2 infectivity between variants and specific mutations on Spike protein*

In H1, it was suggested that Spike protein mutants and SARS-CoV-2 variants could be affected by selected variables. As a result of the analysis, it was found that the 20 variants and mutants were affected by various parameters (Table 2). Therefore H1 cannot be rejected.

Table 2. The results of stepwise regression analysis for different variants (predictors as the independent variables).

Spike Mutation	Predictors and Regression Results
20A.EU2	Predictors: Animal Fat. A regression equation was found (F(1,38)=7.446.,p<.010), with an adjusted R2 of .142 and R2=.164.
20A/S:154K	No meaningful results.
20A/S:439K	Predictors: Urbanization, NDCs, Tax Rates . A regression equation was found (F(3,36)=10.117,p<.000), with an adjusted R2 of .412.
20A/S:478K	No meaningful results.
20A/S:484K	No meaningful results.
20A/S:98F	Predictors: Urbanization, Sunlight . A regression equation was found (F(2,37)=5.166,p<.011), with an adjusted R2 of .176.
20B/S:1122L	No meaningful results.
20B/S:626S	No meaningful results.
20C/S:452R	Predictors: Covid19 Mortalitiy, Anemi, Zinc Deficiency, Conflict Rate. A regression equation was found (F(4,35)=43.256,p<.000), with an adjusted R2 of .813.
20C/S:484K	Predictors: Covid19 Mortality, Conflict Rate, Anemi, Diabet Air Toxicity, Tax Rates, Population Size, Sugar Consumption. A regression equation was found (F(8,31)=51.970,p<.000), with an adjusted R2 of .913.
20C/S:80Y	No meaningful results.
20E (EU1)	Predictors: Sunlight . A regression equation was found (F(1,38)=8.619.,p<.006), with an adjusted R2 of .163 and R2=.185.
20H/501Y.V2	Predictors: Dierra, Household Size, GDP, Lung Cancer, Urbanization. A regression equation was found (F(5,34)=68.963,p<.000), with an adjusted R2 of .897.
20I/501Y.V1	Predictors: Air Toxicity, Iodine Uptake. A regression equation was found (F(2,37)=13.647,p<.000), with an adjusted R2 of .393.
20J/501Y.V3	Predictors: Conflict Rate, Anemi, Zinc Deficiency, Iodine Uptake, Covid19 Mortality, Animal Fat, CO2, COPD, Vegetable Oil, O2 level, Population Size, Diabet . A regression equation was found (F(12,27)=38.240,p<.000), with an adjusted R2 of .920.
ORF1a:S3675	No meaningful results.

S:677H.Robin1	Predictors: Covid19 Mortality, Conflict Rates, Anemi, Diabet, Air Toxicity, Tax Rates, Population Size, Sugar Consumptio. A regression equation was found (F(8,31)=51.970,p<.000), with an adjusted R2 of .913.
S:677P.Pelican	Predictors: Covid19 Mortality Conflict Rates, Anemi, Diabet, Air Toxicity, Tax Rates, Population Size, Sugar Consumption.A regression equation was found (F(8,31)=51.970,p<.000), with an adjusted R2 of .913.
S:H655	Predictors: Conflict Rate, Anemi, Zinc Deficieny, Iodine Uptake, Covid19 Mortality, Animal Fat, CO2, Gini Index. A regression equation was found (F(8,30)=31.829,p<.000), with an adjusted R2 of .866.
S:H69-	Predictors: Rainfall, Sunlight. A regression equation was found (F(2,36)=17.277,p<.000), with an adjusted R2 of .461.
S:K417	Predictors: Dierra, CO2, Lung Cancer, Animal Fat. A regression equation was found (F(4,34)=61.122, p<.000), with an adjusted R2 of .864.
S:L18	Predictors: Gini Index, Diabet, Indoor Air Pollution Deaths, NDCs, Conflict Rates, Anemi . A regression equation was found (F(6,32)=15.309, p<.000), with an adjusted R2 of .693.
S:E484	Predictors: Tax Rates, Anemi, Outdoor Air Pollution Deaths, Gini Index, GDP, COPD, Temperature, Dierra, Covid19 Mortality. A regression equation was found (F(9,29)=48.014, p<.000), with an adjusted R2 of .918.
S:N501	No meaningful results.
S:P681	Predictors: Air Toxicty, Iodine Uptake, Anemi, Lung Cancer . A regression equation was found (F(4,34)=9.134, p<.000), with an adjusted R2 of .461.
S:Q677	Predictors: Population Size, Covid19 Mortality , BMI, Temperature, Vegetable Oil . A regression equation was found (F(5,33)=17.842, p<.000), with an adjusted R2 of .689.
S:S477	Predictors: Cancer . A regression equation was found (F(1,37)= 10.097 p<.003), with an adjusted R2 of .193.
S:Y144-	Predictors: Rainfall, Sunlight, Dierra, Pneume . A regression equation was found (F(4,34)= 14.290, p<.000), with an adjusted R2 of .583.
S:Y453F	Predictors: Lung Cancer, Vegetable Oil . A regression equation was found (F(2,36)= 5.274, p<.010), with an adjusted R2 of .184.

We suggest that these connections in Table 2 between host factors and viral mutants need a carrier inside of the host and it can be a microbiome:

H2: Microbiota is the main carrier of host factors inside the body which specific SARS-CoV-2 mutant is selected by the host.

To test this hypothesis, a Bayesian Network was generated (Figure 3) and some of the outputs of the Network can be represented as Figure 5.

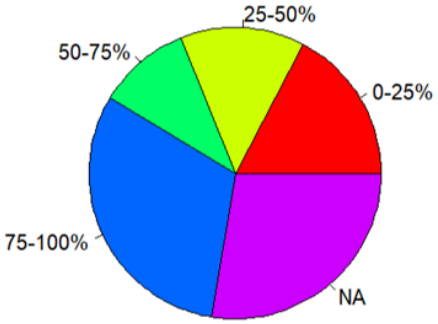

Figure 3. The proportion of variants is explained by independent variables. The separation in the proportions is based on adjusted R squares in Table 2 (The graph is created by R, on RStudio).

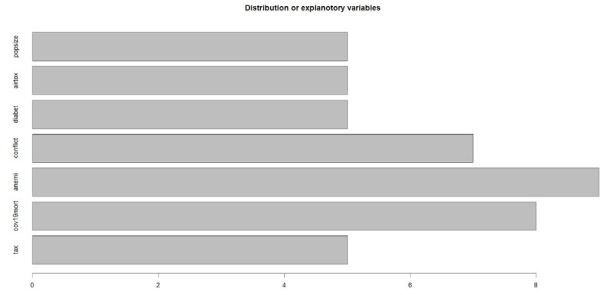

Figure 4. The independent variables of diabetes, tax rates, covid mortality rates, conflict rates, diabetes rates, air toxicity rates, and population size have the most entrants in the regression equations among the variants (The graph is created by R, on RStudio).

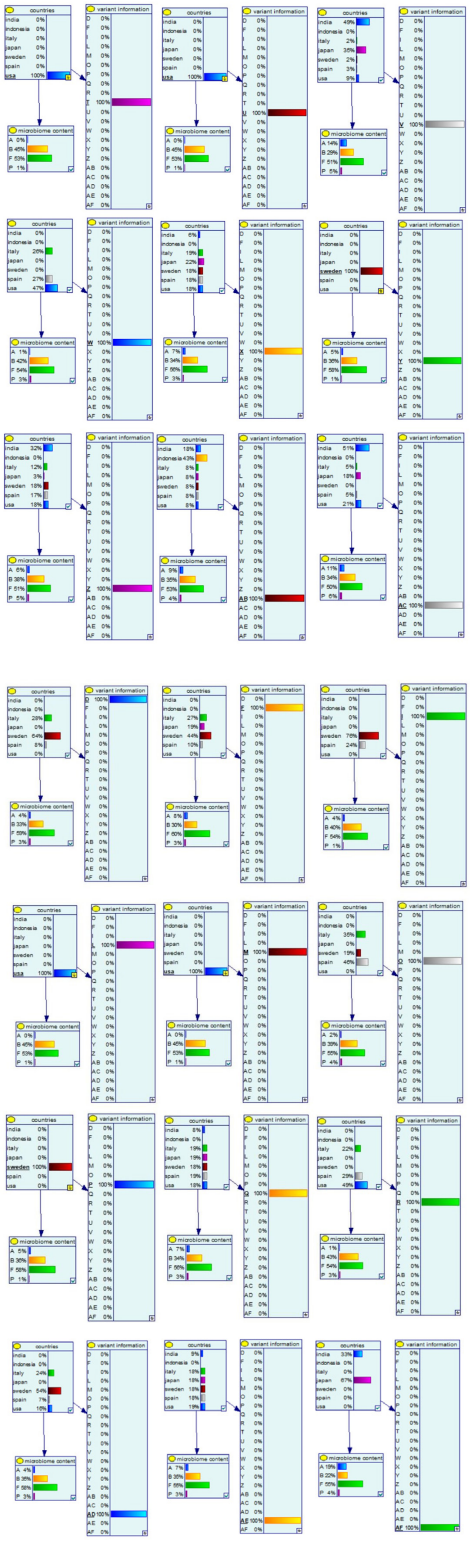

Figure 5. Some outputs of Bayesian Belief Network. *The Microbiome Content Table*: A for Actinobacteria, B for Bacteroidetes, F for Firmicutes, and P for Proteobacteria; *The Variant Information Table*: D for 20A.EU2, F for 20A/S:439K, I for 20A/S:98F, L for 20C/S:452R, M for 20C/S:484K, O for 20E (EU1), P for 20H/501Y.V2, Q for 20I/501Y.V1, R for 20J/501Y.V3, T for S:677H.Robin1, U for S:677P.Pelican, V for S: E484, W for S:H655, X for S:H69-, Y for S:K417, Z for S:L18, AB for S:P681, AC for S:Q677, AD for S:S477, AE for S:Y144-, AF for S:Y453F (The abbreviations are coherent Supplementary Material: 'Data_mutants').

DISCUSSION

Many variants (as dependent variables) were related to the independent variables at various rates (see Table 2, Figure 3). Variants also show various relationships between parameters in the literature. For instance, the 20I/501Y.V1 variant emerged in the United Kingdom and spread globally (49). This variant was predominantly found in Europe. In human reconstituted bronchial epithelium, the 20I/501Y.V1 variant replicates rapidly, contributing to its swift spread (50). This variant is also related to iodine uptake, which is linked to thyroid function. The gut microbiome plays several roles in influencing thyroid function, such as inhibiting thyroid-stimulating hormone (TSH) or modulating the immune response (51). Moreover, Firmicutes and Bacteroides exhibit lower abundance in inflammatory bowel disease (IBD), a condition associated with iodine malabsorption (51). Based on the results in Figure 5, this variant is more dependent on Actinobacteria than other variants. At this point, findings such as that polychlorinated biphenyls (PCBs), a banned air pollutant, reduce the composition of Actinobacteria in the gut microbiota (52) could be used as data to identify a link between this variant and air pollution, as shown in Table 2.

Dietary intake affects the human ACE2 receptor, the main target of the Spike protein, by influencing gene expression (53; 54). Therefore, changes in ACE2 structure due to dietary patterns can be linked to results such as animal fat, vegetable oil, sugar consumption, or malnutrition levels in various countries (see Table 2). Moreover, the mutation S: Y453F (see Table 2) enhances interaction with ACE, facilitating host adaptation (55). Even though studies on the relationship between gut microbiota content and obesity are controversial, there is some evidence that Actinobacteria composition increases in

the gut microbiomes of laboratory animals during obesity (56; 57). Our results show a high correlation between Actinobacteria, which has the highest relative abundance in Japan among the countries studied, and the S: Y453F mutant (see Figure 5). It is suggested that the Japanese diet promotes a healthy gut microbiome (58). Even though the adjusted R-squared value for this spike mutant is not highly descriptive (see Table 2), the strong dependence on Actinobacteria in relation to this mutant (see Figure 5) may be linked to dietary habits that influence the host microbiome. Nevertheless, all these potential links need to be explored more thoroughly.

Moreover, chronic diseases are related to SARS-CoV-2 cases and their severity (59), and our results suggest that diabetes is the most common parameter as a disease among all the variants (see Figure 4). It is well known that SARS-CoV-2 is linked to the economy (60,61), environmental conditions (62-64), and population structure (65, 66), as shown in Table 2. The economic parameters observed in Table 2 could be due to the strong relationship between economic activities and viral diseases (67). Many mutants can be related to different parameters. For instance, the S: Y144 mutation is another Spike protein mutation found in the 20I/501Y.V1 variant and other circulating variants, and it is associated with antibody escape (68). This mutant has been linked to viral shedding in a patient in Washington (69), which is one of the largest metropolises in the United States. This city also experiences deaths due to increasing heat and excessive ozone concentrations (70). In this mutant's regression equation, precipitation and sunlight are included as variables (see Table 2), and it also shows a high level of association with Actinobacteria (see Figure 5). Since gut microbiome composition is influenced by both genetic and environmental factors (71), carriers of environmental factors may be related to the microbiome, especially Actinobacteria. This species is a predominant bacterium in the Italian gut microbiome compared to other nations (24), and infection and death rates from this Spike variant of SARS-CoV-2 are highest in Italy (72). Another example is the S: H69 deletion in the Spike protein, which was sequenced mostly in Europe (73). This mutant occurs alongside others and is another example of immune escape, similar to S: Y144 (74). This mutant can also be associated with Actinobacteria (see Figure 5), suggesting a potential link between antibody escape and Actinobacteria (75) via this Spike mutant. In addition, the higher association rate with Actinobacteria may be related to the widespread use of probiotic supplements, which improve intestinal microbiota, particularly in Europe (76). Moreover, this link cannot be observed solely through Actinobacteria. Firmicutes and Bacteroides are the dominant organisms in the gut microbiome and provide the majority of ecological relations within the human gut microbiota (36). It is possible that mutants with high antibody escape rates may evade host immune defense depending on the presence of these species, as they are associated with immune responses (23,77,78). Therefore, even though some connections exist in the literature, specifying the linkages between these multi-variable systems requires focused research. Additionally, the predictors of this mutant include rainfall and sunlight (see Table 2), so the main factor linking these external factors (sunlight and rainfall) and internal factors (antibody escape and Spike mutants) needs further exploration. The composition of the microbiota, particularly Actinobacteria, may be a mediating factor for the interaction of external and internal forces on the host.

Firmicutes and Bacteroides do not vary much within a certain range, but we see that Actinobacteria shows much more variation

(see Figure 5) depending on each variant. This may be related to the fact that Actinobacteria is a keystone species (22), and the functional relationships of keystone species shape an entire ecosystem. Therefore, changes in Actinobacteria composition may have a more decisive influence on the differentiation of variants than other microbes. It can also be argued that variants and mutants that are not associated with Proteobacteria may be independent of functional diversity in gut microbes, as they show no relation with Proteobacteria. This could be due to the fact that Proteobacteria are responsible for functional diversity in the intestine (36).

This study has limitations. The distributions shown in Figure 5 are dominated by Firmicutes and Bacteroides, while Proteobacteria and Actinobacteria are low, because these four dominant species in the human gut microbiota are present in the host at a certain interval (35). The small size of the data set used was accounted for by the Bayesian method, a probabilistic approach that allows for the interpretation of small data sets. Since the results presented here are the product of a probabilistic approach (see Figure 5), no significant differences are observed. However, the results obtained can help identify links between mutant variations and bacterial compositions. Another issue is that the data used in this study covers the early days of COVID-19. Therefore, much of what this study addresses regarding virulence and spread involves mutants that emerged early in the pandemic. If a study with a broader time interval is conducted, this factor should be taken into account. It is likely that later on, the parameters relevant to COVID-19 and virus mutants may have increased, and the relevance of these parameters and the mutants at hand may have changed. The main point that this study aims to emphasize is that the host microbiota can be, or at least one of the carriers of, external factors within the host's body.

It is known that certain phyla variations are associated with various diseases, particularly in the intestinal microbiota. However, in some cases, variations that are not detected at the phylum level but are detected at the species level are also known to affect host status (79). In this study, the geographic variations observed are at the phylum level, and two dominant phyla (Bacteroidetes and Firmicutes), one keystone phylum (Actinobacteria), and one phylum that influences the functional diversification of the microbiome (Proteobacteria) were analyzed. This is a limitation of the study, as only phylum-level analysis was possible with the available dataset. However, analyses at other levels, such as species or family, may be related to different host metabolic factors and functions. Therefore, researchers who wish to explore this topic should also consider the functional effects at different levels. The existence of a gut-lung crosstalk system (79) may also suggest that different respiratory viral mutants could affect the transmission, virulence, and immune response of the host, as different microbiota compositions are known to influence crosstalk networks. Although the results of this study do not conclusively establish this relationship, it remains a possibility. Since lung microbiota studies are usually conducted in laboratory environments isolated from the organism (41), it may be necessary to perform and investigate such studies at the organismal level.

SARS-CoV-2 is in a highly advantageous position compared to other viruses in terms of both clinical data and the traceability of its mutants globally (48). However, establishing a control group for this study may be necessary to study the viral mutant-microbiota relationship in detail and more meaningfully. In terms of in silico analysis, no comparable data, such as the relationship of COVID-19 with human factors, could be

found for other viruses. Most comparison data remain within the axis of clinical data. Researchers who wish to investigate the viral mutant-microbiota relationship in more detail may consider establishing a comparable control group for the virus.

Additionally, there are challenges in making comprehensive comparisons among microbiome species. Understanding microbiota in terms of composition, diversity, and function is being studied, and it is thought that functional contributions are more important than species diversity in establishing microbiota composition. Ecological microbiota studies seek to understand specific gut microbiota functions in the pathways of host-microbiome interactions. When studying microbial divergence within the microbiota, it is known that there is significant species diversity among humans at the species level. Functional diversity studies, on the other hand, focus on specific genes and functions performed by particular microbial compositions, based on the concept of forming a microbiota community grounded in functional roles within the microbial ecosystem rather than species-level diversity. While microbial composition may vary greatly between individuals in terms of species diversity, there are not significant differences in terms of functionality. In other words, the functional diversity of the human microbiome has been highly conserved among individuals since the core functions in the microbiota play crucial roles in the host's metabolic pathways (80).

However, despite these opposing arguments, this study aims to highlight the potential link between the macro and micro worlds that needs to be explored. One of the most effective ways to investigate this is through a combination of bioinformatics and wet lab processes—identifying indicator microbes and mutants, which can be confirmed by field studies—and conducting comprehensive studies. This approach may help answer the question: What could be the selective forces in a construct that links host factors to the survival of variants? In other words, since external elements need to be maintained inside, and a favorable environment is essential for this, a dynamic system of relationships can be constructed through the internal and external flows of the host. Developing this understanding and collecting and interpreting data in this manner require theoretical frameworks that allow different types of data to be considered on the same plane, rather than merely inferring relationships between macro and micro by combining wet lab and informatics processes.

CONCLUSION

SARS-CoV-2 has advantages in clinical data and mutation tracking compared to other viruses. However, studying the viral mutant-microbiota relationship requires a control group, as existing analyses lack experimental validation. Researchers should consider establishing such a control group. Challenges exist in comprehensively assessing microbiota types, as functional contributions are more crucial than species diversity. Despite significant species-level diversity among individuals, the human microbiome's functional diversity is largely preserved due to key metabolic roles. This study aims to explore the link between macro and micro worlds through a combination of bioinformatics and laboratory processes. Identifying indicator microbes and mutants could clarify selective factors influencing variant survival, emphasizing the need for theoretical frameworks that integrate diverse data types.

ACKNOWLEDGMENTS

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

Funding: This study received no extramural

funding.

Ethical Decleration: Adapted from the dissertation entitled A PROBABILITATIVE EVALUATION OF SARS-COV-2 HOUSING INTERACTIONS IN THE CONTEXT OF META-COMMUNITY AND URBAN ECOLOGY (2021)

Author Contribution: Concept: LNN, SEK, Design: LNN, SEK, Data Collection or Processing: LNN, SEK, Analysisor Interpretation: LNN, SEK, Literature Search: LNN, SEK

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J Immunol Clin Microbiol
Available online at: https://dergipark.org.tr/tr/pub/jicm
DOI: 10.58854/jicm.1510319

Journal of Immunology and Clinical Microbiology

ORIGINAL ARTICLE / ÖZGÜN MAKALE

Effectiveness of Laboratory Tests in Tracking Severely COVID-19 Infections

Şiddetli COVID-19 Enfeksiyonlarının İzlenmesinde Laboratuvar Testlerinin Etkinliği

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Geliş: 04.07.2024, Kabul: 30.09.2024

Abstract

Aim: Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is a virus that causes COVID-19 (Coronavirus Disease 2019), and poses serious difficulties in terms of healthcare systems and global public health. With the large number of publications on COVID-19, clinicians need a synthesis of evidence to provide guidance when dealing with patients with COVID-19. Emerging studies reveal the existence of numerous demographic, clinical, immunological, biochemical and radiographic data that may be useful for clinicians to predict the severity and mortality of COVID-19. The aim of this study; to determine laboratory parameters that can predict the course and severity of COVID-19 disease in patients, independently of the clinic status of selected patients and based on laboratory findings.

Methods: This study is a retrospective cross-sectional study conducted at Karacabey State Hospital between January and April 2022. Among the patients who applied to the COVID-19 outpatient clinic, 469 patients (261 females, 208 males) over the age of 18 and positive for SARS-CoV-2 RT-PCR were included in the study. The patients were divided into groups as outpatients, inpatients and patients in need of intensive care (intensive care unit, ICU). Demographic data (age, gender), COVID-19 RT-PCR results, and simultaneous laboratory parameters of the patients were scanned retrospectively.

Results: When CRP, urea, ferritin, LEU, NEU, MONO, RBC, HGB, HCT, MCV, NLR, PLR, CRP-NR, and SII index values were taken into consideration, a statistically significant difference was found between the groups. Creatinine, ALT, AST, LDH, troponin I, mass CK-MB, D dimer, LYM, EOS, PLT, ELR, and PNR values were not significantly different between the groups.

Conclusion: Advantages of this study; Comparing the changes in the patient's other laboratory findings based on a single positive PCR test result and finding meaningful rates in terms of serious covid risk. The disadvantage of this study is that it is a study independent of the patient's clinic and disease stage. In this study, we found that particularly increased SII was associated with more severe disease progression in patients diagnosed with COVID-19 along with laboratory findings. CRP, urea, ferritin, and indexes such as NLR, PLR, CRP-NR, and SII index values can be used to predict the severity of the disease.

Keywords: SARS-CoV-2, NLR, CRP-NR, SII index

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How to Cite: Yayla E, Korkmaz S, Özmen S. A Effectiveness of Laboratory Tests in Tracking Severely COVID-19 Infections. *Journal of Immunology and Clinical Microbiology* 2024;9(2):89-96

Öz

Amaç: Şiddetli akut solunum yolu sendromu koronavirüs-2 (SARS-CoV-2), COVID-19'a (Koronavirüs Hastalığı 2019) neden olan bir virüstür ve sağlık sistemleri ve küresel halk sağlığı açısından ciddi zorluklar oluşturmaktadır. COVID-19 ile ilgili çok sayıda yayın olması nedeniyle, klinisyenlerin COVID-19'lu hastalarla ilgilenirken rehberlik sağlamak için bir kanıt sentezine ihtiyaçları vardır. Ortaya çıkan çalışmalar, klinisyenlerin COVID-19'un şiddetini ve mortalitesini tahmin etmelerinde yararlı olabilecek çok sayıda demografik, klinik, immünolojik, biyokimyasal ve radyografik verinin varlığını ortaya koymaktadır. Bu çalışmanın amacı; yalnızca SARS-CoV 2 PCR test pozitifliği tanısı almış hastalarda ve seçilmiş hastaların klinik durumlarından bağımsız olarak ve laboratuvar bulgularına dayanarak COVID-19 hastalığının seyrini ve şiddetini tahmin edebilen laboratuvar parametrelerini belirlemektir.
Yöntem: Bu çalışma Ocak-Nisan 2022 tarihleri arasında Karacabey Devlet Hastanesi'nde yürütülen retrospektif kesitsel bir çalışmadır. COVID-19 polikliniğine başvuran hastalar arasından 18 yaş üstü, SARS-CoV-2 RT-PCR pozitif 469 hasta (261 kadın, 208 erkek) çalışmaya dahil edildi. Hastalar ayaktan, yatan ve yoğun bakıma (yoğun bakım ünitesi, yoğun bakım) ihtiyaç duyan hastalar olarak gruplara ayrıldı. Hastaların demografik verileri (yaş, cinsiyet), COVID-19 RT-PCR sonuçları ve eş zamanlı laboratuvar parametreleri retrospektif olarak tarandı.
Bulgular: CRP, üre, ferritin, LEU, NEU, MONO, RBC, HGB, HCT, MCV, NLR, PLR, CRP-NR ve SII indeks değerlerine bakıldığında gruplar arasında istatistiksel olarak anlamlı fark bulundu. Kreatinin, ALT, AST, LDH, troponin I, kütle CK-MB, D dimer, LYM, EOS, PLT, ELR ve PNR değerleri gruplar arasında anlamlı farklılık göstermedi.
Sonuç: Bu çalışmanın avantajları; tek bir pozitif PCR test sonucuna göre hastanın diğer laboratuvar bulgularındaki değişimleri karşılaştırmak ve ciddi covid riski açısından anlamlı oranlar bulmaktır. Bu çalışmanın dezavantajı ise hastanın kliniğinden ve hastalık evresinden bağımsız bir çalışma olmasıdır. CRP, üre, ferritin ve NLR, PLR, CRP-NR ve SII indeks değerleri gibi indeksler hastalığın şiddetini tahmin etmede kullanılabilir.
Anahtar Kelimeler: SARS-CoV-2, NLR, CRP-NR, SII index

INTRODUCTION

At the end of 2019, in Wuhan, China, a virus that belongs to the Coronavirus family which causes severe pneumonia attracted attention because of its contagious and pandemic features. This enveloped RNA virus was associated with the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). The transmission route was found to be the scattering of virus-laden droplets from person to person by coughing-speaking and inanimate surface contact (1). The virus outbreak spread quickly. As of 20 November 2022, over 634 million cases and 6.6 million deaths have been reported from SARS-CoV-2 infection globally (2).

SARS-CoV-2 infection is mostly asymptomatic. The disease can cause insignificant upper respiratory tract infections. However, severe pneumonia, respiratory failure, hyperinflammation, hypercoagulation and death due to multiple organ failure can occur (3,4). Prompt evaluation of clinical and biochemical findings to predict the progression of the disease can lead to the prevention of adverse outcomes (5). Therefore, it is crucial to determine reliable parameters that can predict the severity and mortality of the disease in terms of early intervention and treatment and to deliver health services on a regular and appropriate basis.

According to the recommendations published by the International Federation of Clinical Chemistry (IFCC) COVID-19 Working Group, biochemical and hematological tests in COVID-19 patients may be helpful in the diagnosis of infection-related tissue-organ damage, identifying infected patients at lower risk of severe disease, defining the

patients with a poor prognosis, determining and monitoring the progression of the disease. In this context, the main tests are defined as complete blood count (CBC), D-dimer, PT/APTT, fibrinogen, CRP, ferritin, erythrocyte sedimentation rate (ESR), procalcitonin, troponin, ALT (alanine aminotransferase), albumin, creatinine, urea, LDH (lactate dehydrogenase). These tests have an indicator role in the follow-up of the patients, and also have a prognostic value in terms of the development of the disease and predicting the mortality (6).

The aim of this study is to identify the most relevant biochemical and hematological tests and indices that can help predict poor prognosis and progressive disease of COVID-19.

MATERIALS AND METHODS

This study is a single-centered, retrospective, cross-sectional study conducted at Karacabey State Hospital (Bursa, Türkiye) between January and April 2022. Among the patients who applied to COVID-19 outpatient clinic, 469 patients (261 females, 208 males) over the age of 18 and positive for SARS-CoV-2 real-time reverse transcription polymerase chain reaction assay (RT-PCR) were included in the study. Demographic data (age, gender), COVID-19 RT-PCR results, and simultaneous routine laboratory parameters of the patients were scanned retrospectively from the hospital information management system (HIMS). The patients were divided into groups as outpatients, inpatients, and patients in need of intensive care (intensive care unit, ICU). Following the approval from the T.C. Ministry of Health Scientific Research Platform, permission to conduct this study was obtained from the Bursa City Hospital Clinical Research Ethics Committee (2022-6/3, 20.04.2022).

The detection of SARS-CoV-2 RNA was made in Bursa Public Health Laboratories by the RT-PCR method (outside the institution) (kits used: RTA RT-PCR kit (TÜRKİYE) and diakit (TUSEB-TÜRKİYE), which is the gold standard in the diagnosis of the disease from naso-oropharyngeal swabs. The results obtained from the laboratory information management system (LIMS) system were recorded. CRP, urea, creatinine, ALT (alanine aminotransferase), AST (aspartate aminotransferase), LDH (lactate dehydrogenase), troponin-I, mass creatine kinase-myoglobin binding (CK-MB), ferritin, D-dimer, complete blood count (CBC) parameters (LEU (leukocyte), NEU (neutrophil), LYM (lymphocyte), MONO (monocyte), EOS (eosinophil), RBC (red blood cell), HGB (hemoglobin), HCT (hematocrit), MCV (mean corpuscular volume), PLT (platelet), indices such as NLR (neutrophil-to-lymphocyte ratio), ELR (eosinophil-to-lymphocyte ratio), PNR (platelet-to-neutrophil ratio), PLR (platelet-to-lymphocyte ratio), systemic immune-inflammatory index (SII) and CRP-NR (CRP-to-neutrophil ratio) levels were recorded. The NLR, ELR, PNR, PLR, SII index, and CRP-NR levels were calculated using the relevant data. The SII index was defined as follows: SII: PLTcount×NEU count/LYM count according to the formula described by Bo Hu et al. (7).

CBC was analyzed using Mindray BC-5800 and BC-6000 Hematology analyzer (Mindray Medical International Co. Ltd., Shenzhen, China). The D-dimer was studied in the Diagon with the immunoturbidimetric method (Diagon, Hungary). Ferritin, hs-troponin-I, mass CK-MB measured by chemiluminescence microparticle immunoassay (CMIA) using Abbott Architect I2000SR (Abbott Diagnostics, USA). Urea, creatinine, AST, ALT, LDH (Beckman Coulter, Inc, USA), and CRP (Kinetik Kimya Diagnostics, Turkey) were measured by spectrophotometric assays using Beckman Coulter AU680 analyzer (Beckman Coulter K. K., Tokyo, Japan).

Statistical analysis

The Shapiro-Wilk's test was applied to determine the normality of distribution. The results were presented as median (IQR (interquartile range), minimum-maximum) for continuous variables and numbers and percentages for categorical variables. The One-Way ANOVA test was used to analyze whether the means of group variables differed significantly from dependent variables. Levene's test was used to evaluate the homogeneity of variances between groups. Games-Howell analysis was used to evaluate inhomogeneous variables, and Hochberg's GT2 test was used to evaluate homogeneous variables. $p<0.05$ was used as the significance level. Statistical analyzes were performed in SPSS 24.0 program (IBM SPSS, Chicago, IL, USA).

Results

A total of 469 patients (261 (55,7%) females, 208 (44,3%) males) with positive SARS-CoV-2 RT-PCR results were included in the study. The mean age of all subjects was calculated as 61.37±19.23. (min 18, max 97). In our study, since the measurement of SII index values was retrospectively carried out on patients with RT-PCR positive results, we did not have the opportunity to study the clinical picture and disease severity of the patient. The mean age of male cases with COVID-19 was 63.55±18.83; and 59.64±19.41 years for female cases. Accordingly, the mean age of male and female cases was not significantly different (p>0,5). While the p value was found to be <0.01 for CRP and urea values, the p value was found to be <0.001 for RBC, HGB, HCT and PLR. It was shown that 341 (72.7%) of the patients were followed in the outpatient, 104 (22.2%) in the inpatient, and 24 (5.1%) in the ICU. Gender did not pose a significant risk in terms of hospitalization (p>0,5). The median age of the outpatients was significantly lower than the inpatient and ICU. Patients' age, CRP, urea, creatinine, ALT, AST, LDH, troponin-I, mass CK-MB, ferritin, D-dimer, CBC parameters (LEU, NEU, LYM, MONO, EOS, RBC, HGB, HCT, MCV, PLT), NLR, ELR, PNR, PLR, SII, and CRP-NR data were given in Figure 1 as median, (minimum and maximum), and p values between groups were given in Figure 2.

	Outpatient-Inpatient	Outpatient-ICU	Inpatient-ICU
CRP	**<0,01**	**<0,01**	0,607
Urea	**<0,01**	**<0,01**	**0,04**
Creatinin	0,351	0,585	0,984
ALT	0,834	0,98	0,863
AST	0,535	0,276	0,725
LDH	0,325	0,06	0,181
Troponin I	0,995	1	0,998
CK-MB	0,927	0,82	0,657
Ferritin	**0,036**	**0,038**	0,244
D-Dimer	0,327	0,299	0,391
WBC	**0,031**	0,084	0,74
NEU	**0,001**	**0,006**	0,374
LYM	0,134	0,079	0,624
MONO	0,938	**0,04**	0,097
EOS	0,073	0,106	0,782
RBC	**<0,001**	**0,001**	1
HGB	**<0,001**	**0,047**	0,933
HCT	**<0,001**	**0,037**	1
MCV	**0,003**	0,502	0,956
PLT	0,462	0,426	0,152
NLR	**0,003**	**0,005**	0,076
ELR	0,228	0,408	0,957
PLR	**<0,001**	**<0,001**	0,059
CRP-NR	**0,027**	0,179	0,767
PNR	0,557	0,095	0,585
SII Ind.	**0,002**	**0,047**	0,415

Figure 1: Laboratory findings according to groups, median (minimum-maximum)

(Units are given in parentheses)

	Outpatient	Inpatient	ICU
Age (year)	57(18-97)	75(33-96)	79,5(62-91)
CRP (mg/L)	12,7 (0,8-135,9)	64,1 (2,30-137)	92,6 (5-134,2)
Urea (mg/dL)	26,6 (9,3-390,3)	40,6 (15,1-207,3)	58,4 (35,7-251,5)
Creatinin (mg/dL)	0,84 (0,18-7,94)	0,94 (0,50-5,07)	1,07 (0,21-2,42)
ALT (U/L)	18 (5-286)	18 (6-158)	17 (6-52)
AST (U/L)	22 (10-679)	26 (9-331)	36 (16-161)
LDH (U/L)	213 (113-386)	224 (117-679)	288 (195-896)
Troponin I (ng/L)	4,45 (0,10-17109,10)	16,80 (1,10-3976)	43,05 (3,60-512-40)
CK-MB (µg/L)	1 (0,10-39,70)	1,3 (0,30-14)	2,1 (0,70-12,90)
Ferritin (µg/L)	79,49 (8,71-1111,52)	203,12 (7,25-2000)	522,86 (33,03-2000)
D-Dimer (µg FEU/mL)	500 (220-16600)	804,50 (222-13200)	975 (250-27100)
WBC (10^3/µL)	6,72 (0,85-24,17)	7,24 (0,25-30)	8,66 (1,58-19,23)
NEU (10^3/µL)	4,31 (0,46-16,90)	5,35 (0,01-25,05)	7,02 (0,89-16,83)
LYM (10^3/µL)	1,43 (0,16-18,80)	1,21 (0,23-5,02)	0,89 (0,33-3,80)
MONO (10^3/µL)	0,51 (0,00-1,78)	0,45 (0,01-2,10)	0,37 (0,03-1,41)
EOS (10^3/µL)	0,07 (0,00-4,02)	0,25 (0,00-0,46)	0,00 (0,00-0,14)
RBC (10^6/µL)	4,6 (2,52-7,03)	4,12 (0,44-5,59)	4,2 (2,56-5,20)
HGB (g/dL)	13,3 (6-17,5)	12,1 (6,1-16)	12,5 (7-16,9)
HCT (%)	40,7 (4,09-52,50)	36,85 (5,60-49,30)	38 (21,70-50,90)
MCV (fL)	88,70 (60,30-107,90)	90,20 (71,90-140,50)	91,10 (67,30-103,30)
PLT (10^3/µL)	228 (37-598)	238,50 (49-638)	207 (69-334)
NLR	2,87 (0,26-30,68)	4,42 (0,05-30,10)	6,28 (1,35-28,67)
ELR	0,05 (0,00-3,98)	0,02 (0,00-0,24)	0,00 (0,00-0,23)
PLR	155,79 (9,89-786,96)	170,99 (54,78-1130,43)	229,35 (74,19-1000)
CRP-NR	3,51 (0,20-211,30)	10,07 (0,24-12920)	10,30 (0,73-50,56)
PNR	52,89 (12,13-487,23)	42,81 (10,12-130,2)	35,2 (8,66-130,34)
SII Ind.	685,10 (47,8-7796,9)	924,45 (11,3-8965,6)	1405,7 (145,4-9702,7)

Figure 2: Comparison of laboratory parameters between groups (statistically significant differences (p<0,05) are marked in bold).

There was a significant difference between the three groups in terms of CRP, urea, ferritin, LEU, NEU, MONO, RBC, HGB, HCT, MCV, NLR, PLR, CRP-NR, and SII index values. There was no significant difference between the groups in creatinine, ALT, AST, LDH, troponin I, mass CK-MB, D dimer, LYM, EOS, PLT, ELR, and PNR values.

DISCUSSION

COVID-19 disease is a highly contagious disease with a wide spectrum from subclinical disease to severe pneumonia. Several laboratory tests are used to assess the progression and the prognosis of the disease. Biomarkers related to organ dysfunction, coagulation, inflammation and cytokine storm are changed significantly during the disease (8).

In COVID-19, laboratory tests are used to evaluate the severity of the disease. Significant decreases in LYM and increments in D-dimer and other biochemical and inflammatory markers have been shown in the progression of the disease. Especially the gradual increment in LDH, AST, CK, troponin I and urea are early warning signals for severe disease and poor prognosis (9). The aim of this study was to analyze laboratory data and identify parameters that can predict the severity of COVID-19 disease.

Studies have shown that advanced age is a significant risk factor for hospitalization (10). In our study, similar to the previous studies, advanced age was found to be a significant risk factor for hospitalization. Although literature data shows that the male gender is a risk factor for hospitalization (10), we found no significant difference between male and female patients in terms of hospitalization.

Studies have shown that various biomarkers can be useful in predicting the severity and the course of the disease. Ferrari et al. showed that there is a significant difference in serum LEU, CRP, AST, ALT, and LDH levels between COVID-19 positive and negative patients (11). In their study, Castro-Castro MJ. et al. showed that age, kidney disease, serum creatinine, LDH, CRP, and LYM levels can provide crucial information in terms of the prognosis of COVID-19 disease (12). Aloisio E. et al (13) defined related cut-offs for serum CRP, LDH, D-Dimer, albumin, ferritin, and cardiac Troponin T to aid clinicians in risk stratification and to predict the severity of the disease for hospitalized COVID-19 patients. Singh et al. (14) showed that the decreased levels of serum NEU and LYM levels along with increased CRP and D-dimer levels were associated with disease progression, while moderate changes of these serum biomarkers were seen in patients that had a mild-to-moderate disease. The increment of biochemical markers was related to the severity of the disease. The most significant change was in serum LDH levels; followed by AST, ALT, CK, and creatinine levels, respectively (15). In this study, similar to the previous studies, significant differences were observed in terms of serum CRP, urea, and ferritin values between outpatients and inpatients or ICU. However, we found no difference between the groups in terms of serum creatinine, ALT, AST, LDH, troponin I, and CK-MB levels.

In the studies conducted, the serum LEU and NEU levels were found to be higher in ICU patients compared to non-ICU, while the LYM levels were found to be significantly lower (16-19). Also, the increase in NEU and NLR levels indicates critical illness with a poor prognosis (20). Georgakopoulou et al. (21) reported that lower values of EOS and ELR were associated with worse outcomes and longer duration of hospitalization. The continuous decrease in EOS levels is an indicator of a poor prognosis (22). Similar to these studies, in our study, we found that high levels of serum LEU, NEU, and NLR levels were significantly different between groups. We found no significant difference

in terms of LYM, EOS, and ELR values.

COVID-19 causes hypercoagulation and fibrinolysis abnormalities. Sometimes excessive inflammation causes thrombotic complications such as pulmonary embolism (PE) and disseminated intravascular coagulation (DIC) (15,18,19,23). As a result, abnormal biomarkers of coagulation and fibrinolysis have been linked to poor prognosis in COVID-19 patients. The most typical outcome was an increased concentration of D-dimer. A retrospective study showed a gradual increment in serum D-dimer levels between non-severe, severe, and critical illness (15). In this study, we showed no significant difference between groups.

An innovative marker called SII can predict the prognosis for tumors and other inflammatory diseases of the organism (24). Severe progression can be observed in patients with advanced age and high SII. In these patients, the risk of intubation, mortality, and the need for intensive care is increased (25). In our study, since the measurement of SII index values was based entirely on laboratory studies and was performed by retrospectively screening patients with RT-PCR positive results, we did not have the opportunity to investigate the clinical picture of the patient and the severity of the disease. Similar to previous studies, we found that increased SII was associated with severe disease progression in patients with similar laboratory findings.

Like SII, PLR, a novel indicator of inflammation, reflects the level of systemic inflammation. PLR is found to be associated with malignancies, diabetes, coronary artery disease, and connective tissue diseases (26). In a meta-analysis, it has been shown that the increment in PLR rates is associated with the severe prognosis in COVID-19 patients (27). In this study, our findings were consistent with previous studies in terms of PLR rates.

The limitations of this study are that the study was performed in a single center, the number of patients included in our study was relatively low, the viral load of the patients was unknown and the comorbid diseases of the patients were not taken into account.

CONCLUSION

The advantageous aspects of this study are that proportional data that can determine the course of the disease are obtained by using measurable laboratory result values of patients in laboratory procedures. In this respect, significant results may be guiding in serious viral infections with similar clinical features. The disadvantages of the study are; being a retrospective evaluation, interpretation of test results being based on PCR positive test results; the only standard parameter is the selection of reference intervals. We did not have any data regarding the clinical conditions (comorbidity status) of the patients, the medications used in the patients' information, the treatment protocols applied to the patients, and at what stage of the disease blood samples were taken from the patients. In this study, we found that there was a significant difference between the three groups in CRP, urea, ferritin, LEU, NEU, MONO, RBC, HGB, HCT, MCV, NLR, PLR, CRP-NR and SII index values. There was no significant difference between the groups in creatinine, ALT, AST, LDH, troponin I, mass CK-MB, D dimer, LYM, EOS, PLT, ELR and PNR values. A gradual and significant increase was observed only in urea values in the outpatient, inpatient and intensive care groups. Monitoring markers of severity can help clinicians identify and monitor patients at increased risk of progression to severe COVID-19 infection and similar viral infections. These key biomarkers may help in the early detection of serious diseases. Further research is needed to determine the role of these biomarkers in the clinical progression of COVID-19 patients.

ACKNOWLEDGEMENT

Conflict of Interest: The authors declare that there is no conflict of interest.

Ethics Committee Approval: The study was approved by Bursa City Hospital Clinical Research Ethics Committee (2022-6/3, 20.04.2022).

Financial Disclosure: The authors declared that this study has received no financial support.

Peer-review: Externally peer-reviewed.

Authorship Contributions: Concept – S.K., E.Y.; Design – S.K., E.Y.; Supervision – S.K., E.Y., S.Ö; Funding – S.K., E.Y., S.Ö; Materials – S.K., E.Y., S.Ö; Data collection &/or processing – S.K., E.Y., S.Ö; Analysis and/or interpretation –S.Ö; Literature search – S.K., E.Y.; Writing – S.K., E.Y., S.Ö; Critical review – S.K., E.Y., S.Ö

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J Immunol Clin Microbiol
Available online at: https://dergipark.org.tr/tr/pub/jicm
DOI: 10.58854/jicm.1389854

CASE REPORT/OLGU SUNUMU

Epididymo-Orchitis Caused by Hand, Foot, and Mouth Disease In Adults

Erişkinde El Ayak Ağız Hastalığına Bağlı Epididimoorşit

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Geliş: 04.04.2024, Kabul: 30.09.2024

Abstract

Hand, Foot, and Mouth Disease (HFMD) is predominantly recognized as a childhood disease and is not generally anticipated to manifest in adults. In addition to inducing symptoms such as fever, herpangina, and distal-extremity rashes, HFMD can also lead to rare complications such as encephalitis and onychomadesis. Epididymo-orchitis, however, is not considered an expected complication of HFMD.

In our case, a patient diagnosed with HFMD presented with testicular pain four days later, and epididymo-orchitis was subsequently identified in the patient initially suspected of testicular torsion. Cases of Coxsackie virus-related epididymo-orchitis have been reported in the literature, and these cases can be mistaken for torsion, thereby posing the risk of unnecessary surgical interventions. Testicular pain in a young adult with a history of HFMD should be considered a potential diagnosis of viral epididymo-orchitis.

Keywords: Hand, Foot, And Mouth Disease, Epididymo-Orchitis, Coxsackie Virus

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How to Cite: Gültekin AC. Epididymo-Orchitis Caused by Hand, Foot, and Mouth Disease In Adults. *Journal of Immunology and Clinical Microbiology* 2024;9(2):97-100

Öz

El, Ayak ve Ağız Hastalığı (HFMD), ağırlıklı olarak çocukluk çağı hastalığı olarak kabul edilmektedir ve genellikle yetişkinlerde ortaya çıkması beklenmemektedir. HFMD, ateş, herpanjina ve distal ekstremite döküntüleri gibi semptomları tetiklemenin yanı sıra ensefalit ve onikomadezis gibi nadir komplikasyonlara da yol açabilir. Ancak epididimo-orşit, HFMD'nin beklenen bir komplikasyonu olarak görülmemektedir.
Bu olguda HFMD tanısı alan bir hasta dört gün sonra testis ağrısı şikayetiyle başvurmuş ve testis torsiyonundan şüphelenilen hastada daha sonra epididimo-orşit tespit edilmiştir. Literatürde Coxsackie virüsüne bağlı epididimo-orşit vakaları bildirilmiş olup, bu vakalar torsiyon ile karıştırılarak gereksiz cerrahi girişim riski oluşturabilir. HFMD öyküsü olan genç bir yetişkinde testis ağrısı, potansiyel bir viral epididimo-orşit tanısı olarak düşünülmelidir.

Anahtar Kelimeler: El, Ayak, Ağız Hastalığı, Epididimo-Orşit, Coxsackie Virüsü

INTRODUCTION

Hand, Foot, and Mouth Disease (HFMD) is a viral eruptive disease primarily caused by Coxsackie A16 and Enterovirus 71. The disease mostly afflicts children under 10 years of age and is rarely observed in adulthood, probably due to the safeguarding effect of cross immunity from previous enterovirus infections. The most common clinical findings encompass fever, herpangina, and distal-extremity rashes (1). Rare sequelae such as onychomadesis and encephalitis may manifest in the aftermath of the infection (2, 3).

Epididymo-orchitis is a disease usually observed in young men, often attributed to Chlamydia trachomatis and Neisseria gonorrhoeae (4). Mumps, Influenza, HIV and the Coxsackie viruses have been identified as causative agents of epididymoorchitis in adult men (5).

HFMD is predominantly recognized as a childhood disease and is not generally anticipated to manifest in adults. Epididymo-orchitis is not among the expected clinical findings in HFMD. In this case, we present a rare complication in adults, a demographic that is uncommon for HFMD. Our objective is to depict a case of epididymo-orchitis attributed to HFMD in the adult population.

CASE REPORT

A 34-year-old male patient, without any known comorbidities and a surgical history limited to appendectomy, presented with complaints of widespread muscle pain that started 2 days prior, accompanied by redness in the throat, fever exceeding 39 ℃, and rash on his hands and feet (Figure 1).

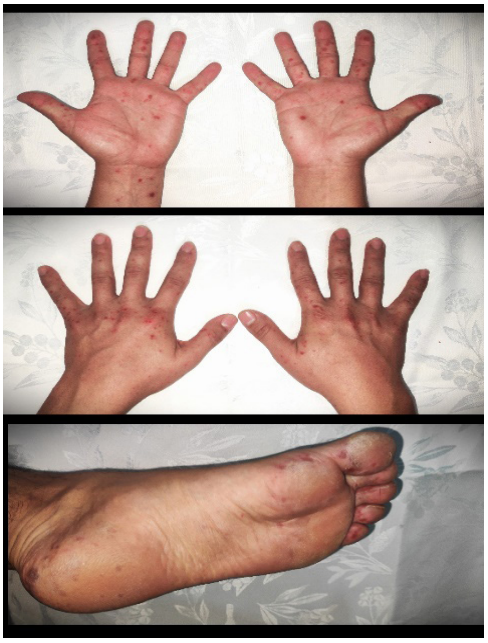

Figure 1. Rashes on hands and foots

In the history, it was ascertained that the patient's son was diagnosed with HFMD four days prior. Given the presence of typical symptoms and rashes, coupled with the recent identification of the same disease in his son, he was diagnosed with HFMD without the need for additional testing. The patient was advised to undergo symptomatic treatment and adhere to isolation measures. Three days later, the patient presented with radiating pain from both testicles to the inguinal region, more prominent on the right side, and a fever of 40°C. The patient was referred to the emergency room with suspicion of testicular torsion. Doppler ultrasonography revealed epididymo-orchitis bilaterally. There was no suspicious sexual intercourse or urinary tract infection in the patient's history. Coxackie A IgM was tested to confirm the diagnosis and was found to be positive. The patient was considered to have epididymo-orchitis due to Hand, Foot, and Mouth Disease. Testicular elevation, analgesic and antipyretic therapy, and cold application were advised to the patient. After a 7-day antibiotic-free follow-up, the patient's symptoms alleviated, and follow-up ultrasonography indicated regression of epididymo-orchitis.

CONCLUSION

HFMD is predominantly recognized as a childhood disease. Consequently, transmission to adults is frequently overlooked by both patients and healthcare professionals. In adults, epididymo-orchitis is commonly attributed to bacterial factors, usually arising after suspected sexual contact or urinary tract infection. Post-viral epididymo-orchitis is often associated with mumps (6). It may not be considered that such a complication may ensue following HFMD.

In 2014, Vuorinen et al. demonstrated that Coxsackie A virus, isolated for the first time from the epididymal fluid in a case undergoing surgery for testicular torsion, could induce epididymo-orchitis, highlighting the potential risk of unnecessary surgical interventions (7). In the case report of Di Lella et al. (2021) reported a case with testicular mass and viral epididymo-orchitis attributed to HFMD (8).

In this case, it is aimed to emphasize the occurrence of HFMD in adults and the potential detection of epididymo-orchitis as a consequence. In conclusion, contemplating the diagnosis of viral epididymo-orchitis in individuals with recent HFMD history presenting symptoms of testicular pain and swelling will mitigate unnecessary surgical interventions and inappropriate management.

ACKNOWLEDGEMENT

Conflict of Interest: The authors declared no conflict of interest regarding this article.

Financial Support: No financial support was used by the authors during this study.

Ethical Statement: Informed consent was obtained from the participant and the rules of the Declaration of Helsinki were followed to conduct this study.

Author Contributions: Concept: ACG, Design: ACG, Supervision: ACG, Instrumentation: ACG, Data collection and processing: ACG, Analysis and interpretation: ACG, Literature review: ACG, Writing: ACG, Critical review: ACG

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