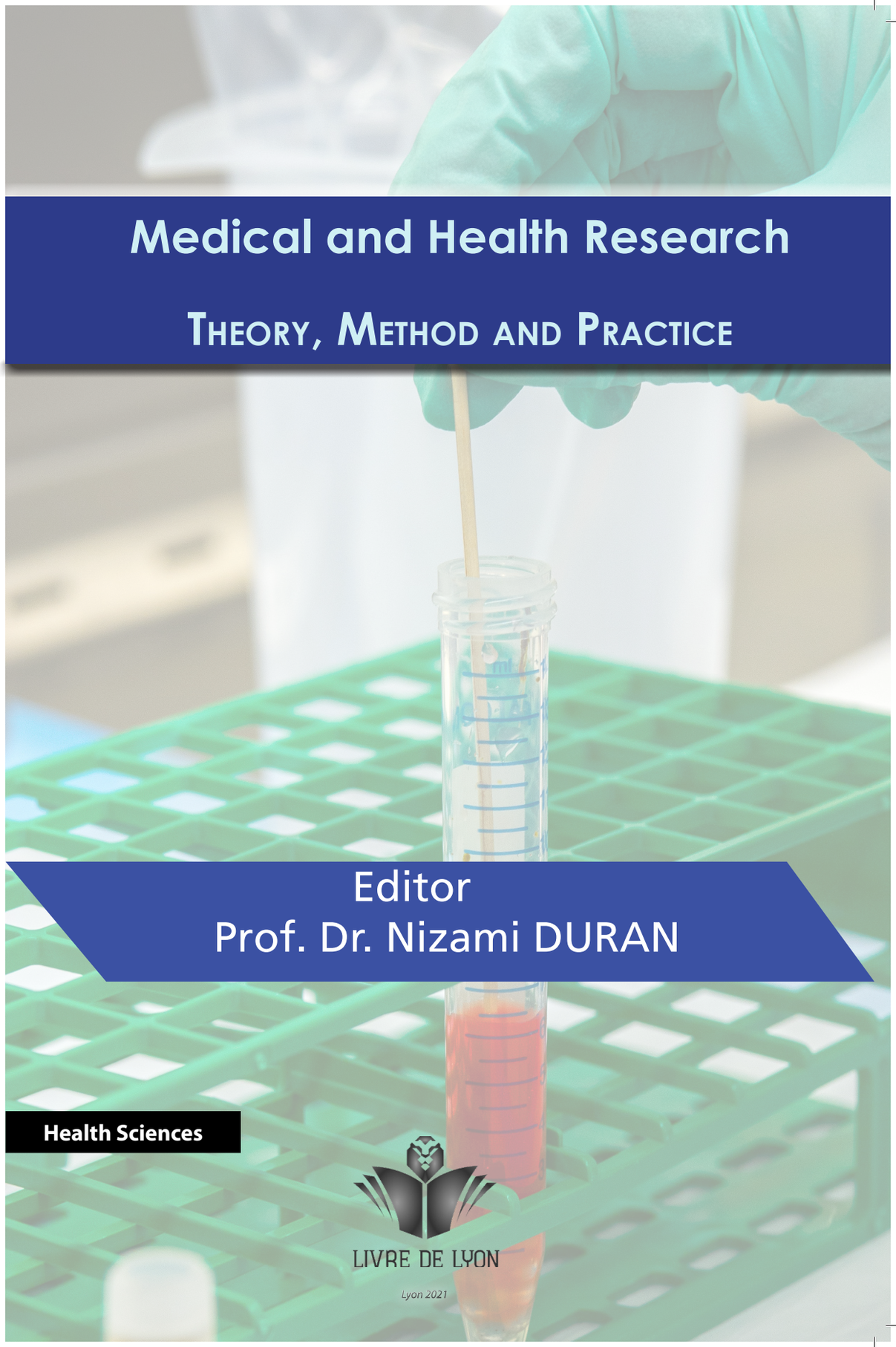
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Prof. Dr. Nizami DURAN

Health Sciences

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PREFACE

Dear reader,

This book contains preclinical and clinical studies in many fields of medicine, and I think that it will make important contributions to your personal development in your field. This book aims to assist health scientists in planning and conducting research. The topics covered in this book are current issues and will contribute to your perspective. It will be seen that important experiences are presented in a wide variety of issues in the field of health, and it also includes innovations in diagnosis and treatment approaches. It will be an important reference that I think can be a very useful guide or reference book that both health students and health professionals, as well as health educators, can benefit from.

I would like to express my deepest gratitude to all the authors who contributed to the preparation of this book.

Prof. Dr.Nizami DURAN
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CHAPTER IX

MOLECULAR APPROACHES TO THE COVID 19 IN RESPIRATORY AND IMMUNE SYSTEMS

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1. Introduction

The coronavirus family are single-stranded, positive-polarity, non-segmented, enveloped pleomorphic viruses with the largest RNA genome. The large genome causes the virus to be less dependent on the host cell during replication. Its replication takes place in the cytoplasm of epithelial cells of the respiratory system and gastrointestinal tract. The term "corona" means crown in Latin, and the virus takes its name from the crown-like structures in its structure. In general, the genome size of RNA viruses is usually less than 10 kb, since the mutation rates in the replication of RNA viruses are much higher than that of DNA viruses. However, the coronavirus genome has the largest genome among RNA viruses and is 30 kb in length (1-4).

Coronaviruses belong to the order Nidovirales, family Coronaviridae, subfamily Coronavirinae. The subfamily contains four genera within which they are denoted by Greek letters: Alphacoronavirus, Betacoronavirus, Gamakoronavirus and Deltacoronavirus. Only alpha and beta coronaviruses are known to infect humans. Bats are the natural host of coronaviruses, and their development takes place in bats and it is accepted that most human coronaviruses are derived from bat reservoirs. The genetic similarity of bat betacoronavirus belonging to SARS-CoV-2 and subgenus Sarbecovirus has been confirmed by various research groups. While there was 96.2% similarity between the sequences of bat SARS-associated coronaviruses (SARSrCoV; RaTG13) collected in Yunnan province, China, and the entire sequence of the new virus,

this degree was compared with the genomes of SARS-CoV (approximately 79%) or MERS-CoV (approximately 50%). No similarity was found (5-7). In the genomic sequence analyzes of SARS-CoV-2 viruses isolated from some patients, the fact that the sequence similarity was found over 99.9% is the most important indicator of the transmission of the virus to humans (8-11). However, the difference in the incidence of adults or children among humans makes one think about the sequence difference between them. Is it the lack of growth factors and their gene sequences in adults? What are the gene sequence differences between adults and children, I think some issues need to be investigated. In this regard, genetic sequence analyzes may do more intensively and examined.

2. Immune system related to Corona virus structure

The protein structures that make up the crowned structure of the coronavirus are known as S proteins. S protein; It is in the form of protrusions on the virus surface, on the viral envelope, and provides attachment of the virus to the host cell by binding to the receptor and membrane fusion. It is an important viral protein that determines host cell tropism. S protein has S1 and S2 loops S1 protein is responsible for host cell receptor binding while S2 protein is responsible for membrane fusion. The S2 protein of 2019-nCoV shows 93% similarity with bat-SL-CoVZC45 and bat-SLCoVZXC21. In the S1 protein, this similarity is approximately 68%. Both the N and C terminal portions of the S1 loop can bind to the host cell receptor. Although 2019-nCoV and SARS-CoV are in different tribes, both viruses have 50 conserved proteins in the S1 loop. This result; suggests that the new coronavirus may also use angiotensin 12 COVID-19 converting enzyme 2 (ACE 2) as a receptor, as in SARS-CoV. Apart from the S protein, M, E, N and Hemagglutinin Esterase Proteins are also proteins in the coronavirus structure. Among these proteins, the N protein differs from the others in terms of location and function. N protein; together with the M protein, they are envelope proteins that play a very important role in virus formation and release. It contains two domains that can bind the viral genome through different mechanisms. This protein binds to the nsp3 protein to help the genome bind to RTK and contributes to virion formation. It plays a role in the regulation of replication and transcription of viral RNA. The N protein also acts as an interferon antagonist, thus inhibiting the attempt to destroy the virus by the immune system (12). While our immune system defends against the virus, the N protein of the virus prevents it. In this case, even if our defence system is strong, we can sometimes succumb to the virüs. The way to prevent this is to consume N protein-inhibiting drugs, foods, and to detect such inhibitors from plants. Researchs can do on this subject. N protein gene expression RT-PCR studies can be performed. Does the effect of N protein against the functioning

of our immune system cause it to cause more serious, unavoidable, dangerous and threatening consequences for human health, which distinguishes the coronavirus from others? This may include the answer to why we don't get over the coronavirus like a cold or flu.

3. Immunostimulatory agents in coronavirus

It is known that vaccines are used for the formation of immune response specific to antigens such as viruses in metabolism, that is, for stimulating the immune system (13). Biological agents or synthetic, organic substances are used in vaccines. However, the contribution of phytochemicals in naturally sourced foodstuffs to the immune system has been investigated. Many immunostimulatory plants have been found in the studies. These include many plants such as Actinidia macrosperma, Allium sativium, Aloe vera, Aesculus indica, Andrographis paniculata, Asparagus racemosus, Baliospermum montanum, Curcuma longa. Immunostimulatory effects of different extracts of ethanol, methanol and distilled water from parts of these plants such as roots, leaves, flowers and fruits were determined. It is recommended that they are widely used as drugs in autoimmune diseases, allergic conditions, cancers and virological infections (14-16).

4. Immunomodulatory agents in coronavirus

Immunomodulators are known as biological or non-biological agents that can affect the immune response, which represents the activated reaction sequence that changes the immune response of the host, to protect the organism against infective agents, environmental injury and disease (17-19). There are many herbs that modulate the immune system. There are many studies about the effects of herbal extracts prepared in different solvents such as methanol and water on the immune response. (20-25). Seaweeds are not only rich in polysaccharides, minerals, and certain vitamins but they also contain bioactive substances such as proteins, lipids, and polyphenols. These bioactives are known to possess potent antibacterial, antiviral, and antifungal properties. C. crispus has antiviral properties and is said to be particularly useful for dislodging mucus (26). In the treatment of severe Covid 19, immunomodulatory synthetic drugs such as antimalarials, anti-IL6, antiIL-1, calcineurin and JAK inhibitors, corticosteroids, immunoglobulins, heparins, angiotensin-converting enzyme agonists and statins are used (27). In many international clinical studies, it is also known that vitamin D has positive immunomodulatory effects against covid 19 (28). In the later stages of Covid 19 disease, systemic proinflammatory cytokines and biomarkers are elevated and show worse correlation and reduced survival chances. Here, immune modulators come into play and inhibit cytokines and

treat cytokine storm (29). A rapid and strong increase of IFNa/β is required to inhibit dsRNA replication of the Covid 19 virus. To allow efficient production of IFNa/β, as well as the synthetic drugs used for this, it is necessary not only to alleviate a TGFβ-dependent brake but also to strengthen its production. For this, 1,8-cineole, a small, aromatic and plant-derived molecule, can be used and many similar plant molecules can be discovered (30).

5. Respiratory system in coronavirus

The main cause of various viruses seen in the last 20 years is by members of the Betacoronavirus genus. SARS-CoV and MERS-CoV viruses, which emerged in 2002-2003, cause severe acute respiratory syndrome and viral pneumonia. This syndrome is a respiratory failure characterized by widespread inflammation of the lungs with symptoms such as shortness of breath, rapid breathing, and bluish skin colour (31). In Covid 19, which was seen as of 2019, many and different symptoms were seen compared to SARS and MERS (32). The especially long and severe acute respiratory syndrome may increase the risk of arterial or venous thromboembolism (33). Mucus secretion and the effect of mucociliary clearance play an important role in preventing viruses taken from the nose through the airway epithelium from damaging the epithelial tissue before reaching the lung. Inhaled virus particles probably also infect different epithelial cell types on their way to the distal lung. It is understood that the first viral contact in the nasal mucosa occurs by binding the viral S protein to the ACE2 (angiotensin-converting enzyme-2) receptor and then cleavage of the S protein by TMPRSS2 (transmembrane serine protease 2) (34-36). Many immunostimulating agents are needed to combat so many symptoms. These include currently used vaccines and drugs (37). However, there are studies on the use of natural substances as food and beverage. In addition, 179 plants are recommended to be used in the treatment of Covid 19. The fact that phytochemicals such as β-sitosterol, stigmasterol and quercetin are related to the antiviral signalling pathway indicates that plants containing them can also be used in the treatment of covid 19 symptoms (38-40).

6. Real Time RT-PCR studies in coronavirus

The first test method detected at the time the virus emerged in 2019 was the nasopharyngeal swab test containing RT-PCR. The reliability of the negative result of this test is also low (41). However, a real-time RT-PCR nasopharyngeal swab test is used in the 2019 novel coronavirus detection and laboratory diagnosis study (42, 43). In January 2020, the World Health Organization focused on research on more reliable covid 19 detection tests than real-time RT-PCR. It has been suggested that various methods should be used to evaluate

the proposed tests. Dogs, in particular, have been successfully trained and used to detect diseases in humans. For Covid 19 detection, they used explosive detection dogs and after training them to smell COVID-19 in the sweat of patients, these dogs were able to successfully screen 3249 people who tested negative for SARS-CoV-2 from a cohort of 3290 people. In addition, using Bayesian analysis, the sensitivity of the K9 test was found to be superior to the RT-PCR test performed on nasal swabs from a cohort of 3134 individuals. Given its high sensitivity, short turnaround time, low cost, less infestation, and ease of administration, detection dog testing is emerging as a better alternative to RT-PCR in screening for SARS-CoV-2 in asymptomatic individuals (44-46). In another study, a comparison of the basic analytical and clinical performance of RT-PCR kits produced and released to the market by many different companies at the end of 2019 was included. It was concluded that all RT-PCR kits evaluated in this study can be used by experienced molecular diagnostic laboratories for routine diagnosis of COVID-19 in patients (47). In addition to these, there are applications of RT-PCR test by developing different methods. In one study, four virus inactivation methods were used for the rapid detection results of COVID-19 nucleic acid. The results of the four treatment methods and specimens without inactivated treatment have shown good consistency (48). In another study, a novel antigen-based rapid detection test (RDT) for the diagnosis of SARS-CoV-2 was evaluated in respiratory samples. The RDT assay has the potential to become an important tool for the early diagnosis of SARS-CoV-2, particularly in situations with limited access to molecular methods (49). There is even a study in which it was applied in serological tests for severe acute respiratory syndrome coronavirus 2 (50).

7. Conclusion

Human body systems have different metabolic activities. This is why many diseases and COVID-19 affect different people in different ways. Most infected people develop mild to moderate illness and can recover without hospitalization. In other patients who do not recover, two important metabolic systems play a role. These are the immune system and the respiratory system. In this study, evaluations were made about the quantitative molecular changes and the status of RT-PCR studies, the effects of coronavirus on the immune and respiratory system, from the onset of Coronavirus 2019 to the present. Expression of molecules that will provide defence against quantitative molecular increases and changes in these systems will change the course of the disease. There are substances of synthetic and natural origin that must be imported to support the defence. Thanks to these agents' immunomodulatory and immunostimulating effects, molecular changes in metabolism will positively change the course of

covid 19 diseases. And also, in this study, the development studies carried out in the real-time RT-PCR method used in virus detection. During the period from the beginning of the virus in 2019 to the recent times are included. Because, since 2019, there are many studies in the literature on the real-time RT-PCR method and its development, which is used in the detection and laboratory diagnosis of the Coronavirus. However, studies using agarose gel electrophoresis molecular techniques and quantitative gene expression studies by RT-PCR are rare. We hope that scientific studies that will fill the gap on this subject will enlighten the unknowns about coronaviruses.

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