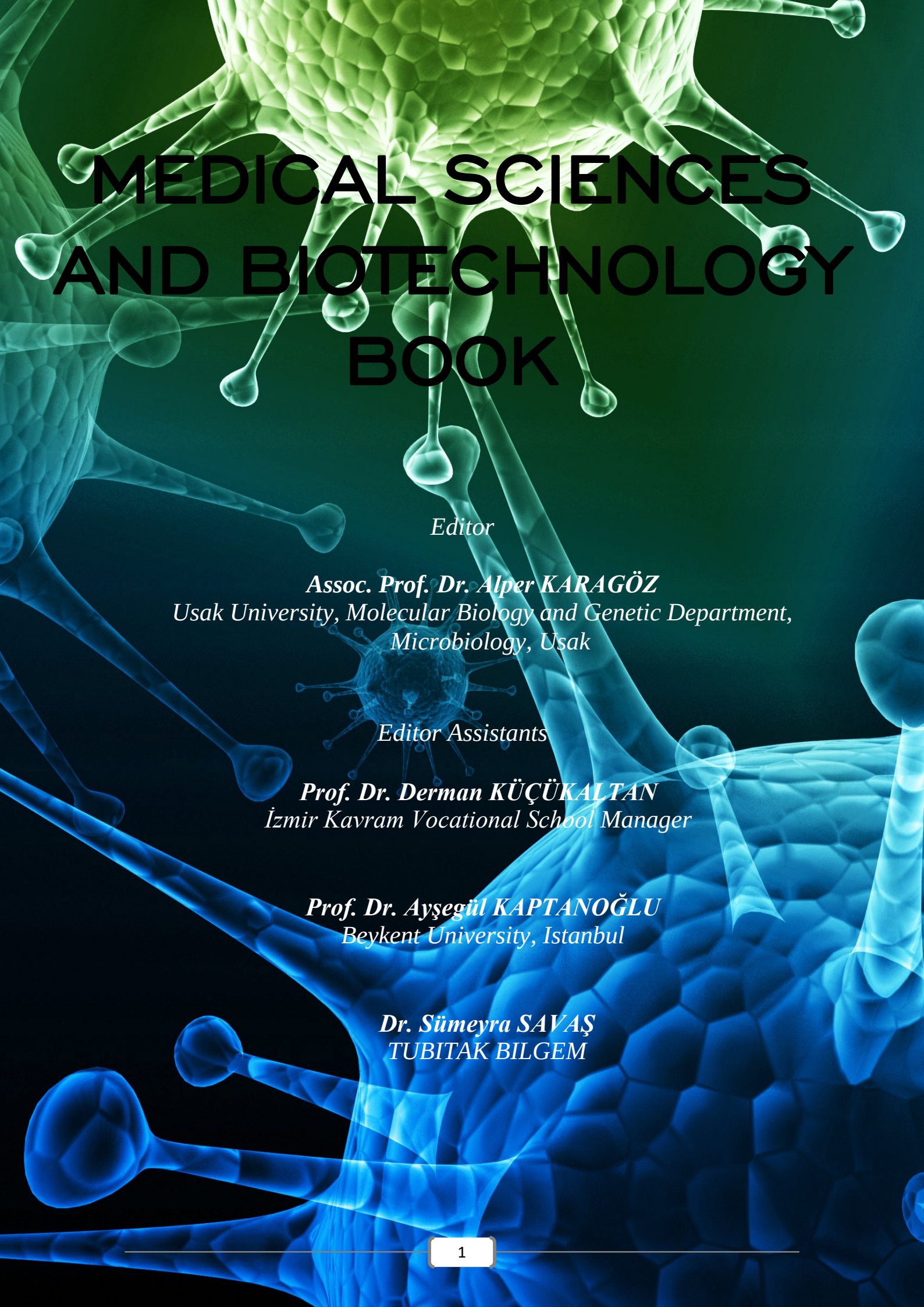

MEDICAL SCIENCES AND BIOTECHNOLOGY BOOK

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PREFACE

Medical Science and Biotechnology book which was created with great devotion, experiences and valuable works of academicians has been published. I would like to thank our authors who contributed to the publication of this book, to administration of Uşak University, Beykent University, and İzmir Kavram Vocational School for their support, and of course to our students who will carry us to the future, as well as to my wife Öznur Karagöz and my daughters Işıl and İnci for their support and encouragement.

Yours sincerely.

Assoc. Prof. Dr. Alper KARAGÖZ

October, 2020

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Hande AYTUĞ (Usak University)

Covid-19 Laboratory Diagnosis

Alper KARAGÖZ

Coronaviruses are a large family of viruses that cause more severe diseases such as the Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS). The coronavirus, which was not identified in humans before, first appeared in Wuhan, China in December 2019, causing a pandemic all over the world. This virus, which is a pandemic factor, was named COVID-19 by the WHO (World Health Organization) (1). There is no vaccine and treatment developed for Covid-19 yet. Therefore, reliable laboratory diagnostics are needed to reduce the spread of this virus and to identify and isolate infected individuals. The diagnosis is made according to clinical laboratory and radiological findings. Due to the non-specificity of Covid-19 symptoms and radiological findings, virus diagnosis requires confirmation by nucleic acid-based polymerase chain reaction (PCR) that replicates a specific gene sequence of the virus. WHO prepared a guide for the treatment of suspected cases on March 19, 2020 (1,2). There are two basic principles in screening tests. In one of these, the virus itself, and in the other, the host organism's response to the virus is tried to be determined. It has been observed that serological tests based on antigen and antibody detection may give false positive results due to cross-reaction (5). For this reason, the real-time reverse transcriptase (real time reverse transcriptase) polymerase chain reaction (RT-PCR) -based method continues to be the most effective laboratory diagnostic test for the diagnosis of Covid-19 worldwide (3).

Molecular Diagnostic Methods:

For the detection of Covid-19, molecular methods are applied using sequence analysis and PCR techniques based on genomic analysis. The genome sequences of a large number of Covid-19 viruses are extracted with the technique of sequence analysis. Based on these results, epidemiological studies, verification of erroneous RT-PCR results or mutation studies can be performed. However, this test is not a preferred test because it is not practical. Currently, the most valid test in terms of time has been determined as RT-PCR (4).

Approximately one week after the first diagnosis of the disease was determined, a PCR-based study was carried out by German scientists using the throat and nose swab samples of a patient, and in this way, the first Covid-19 diagnosis protocol was presented to the scientific world by WHO (2). According to this protocol, it was determined that the SARS virus, which is in the same family as SARS-CoV-2, has similar genomic characteristics. The main goal of the study is to see the E gene belonging to SARS-CoV-2 and the RNA polymerase enzyme gene linked to RNA. Later, in another protocol published by the US Center for Disease Control and Prevention (CDC), he developed his own test by detecting the RNA-linked RNA polymerase gene with three separate genomic sequences in the N gene in the capsid structure of the virus (2). The principles of these two tests are the same, but their genetic goals are different. It is based on the detection of nucleic acid sequences belonging to the virus, such as real-time reverse transcription polymerase chain reaction (rRT-PCR), which is confirmed by nucleic acid sequencing when cases need to be confirmed. Although new protocols targeting N, E and S genes for molecular tests have been published, it has been observed that a simpler algorithm, such as a screening test with a single descriptive targeted rRT-PCR, is sufficient (11). According to this approach, real-time PCR (qPCR) (RT-qPCR) targeting the RdRp gene fragment should be studied in reference laboratories determined by the Ministry of Health in our country. The most important advantage of this test is that it provides analysis of thousands of samples in a single day and has a test sensitivity of 95% (7,8). The disadvantages are; False positive results can be given by cross-reacting primers with nucleic acids resulting from

infection with other viruses and bacteria. In this case, the detected agent may not be the exact cause of the disease.

One of the most important issues affecting the reliability of the nucleic acid amplification test is the sample shape. Swab samples taken from the nasopharynx or oropharynx and bronchoalveolar lavage fluid and sputum samples are used as samples for virus isolation. Sputum and tracheal aspirates, which are among the lower respiratory tract samples, are not highly recommended due to the risk of aerosol production (6). Depending on the stage of the disease, the presence of viruses in samples taken from these regions may also vary. The detectable viral load depends on the days after the onset of the disease. Viral loads were reliably determined within 14 days after the onset of the disease. Considering the variations in viral loads, a negative result does not mean that the disease is absent. The reasons for this can be attributed to the wrong sampling, low viral load or mutated genome. When looking at positive test results related to viral nucleic acid detection in patients, bronchoalveolar lavage (BAL) was 94%, bronchoscopy biopsy was 46%, sputum 72%, nasal swap 63%, pharyngeal swap 32%, stool 29%, blood 1% and urine 0%. (9).

Apart from the most widely used real time RT-PCR analyzes for Covid-19 diagnosis, isothermal amplification, CRISPR, next generation sequencing (NGS), micro NMR (μNMR) analyzes are also used. Although the search for new techniques for the diagnosis of Covid-19 continues, the most suitable standard method is the real time RT-PCR-based nucleic acid amplification test and sequencing analysis (2,10).

As a result, accurate and reliable test results make it possible to prevent the disease, to get it under control quickly and to provide the necessary support to the patient in a timely manner. Therefore, the suitability of the diagnostic tests, the people and the time of these tests should be determined by organizations such as WHO. The tests determined in the diagnosis of SARS-CoV-2 may vary depending on the sample type, test protocol and the mutation of the virus. For this, there is a need for reliability analysis with existing data. Potential mutations that may occur in SARS-CoV-2 in PCR-based tests used for diagnosis may cause erroneous results, and for this, the entire genome sequence of the virus isolated from positive patients should be regularly made. It should also be checked whether the primer and probe sequences used in these tests are appropriate. Although it is seen that there is no problem in conducting the studies in theory, it is known that the most important problem is time. Therefore, increasing the number of tests and shortening the time to give the test result provides better management of the process. However, limited molecular testing capacities and the inadequacy of manufacturers to develop diagnosis are among the problems that arise. In order to control the disease, fast, reliable and economical test kits are needed.

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Immunomodulatory Effect of Mesenchymal Stem Cells in Respiratory Tract Infections Caused by Covid-19

Aynur KARADAĞ GÜREL

Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) is the pathogen that causes 2019 coronavirus disease (COVID-19). The COVID-19 outbreak caused 942 735 deaths worldwide, and the pandemic has grown rapidly. It has become important to consider various treatment options to effectively treat people around the world. Available data suggest that severely ill patients tend to have a high concentration of pro-inflammatory cytokines compared to moderately ill patients, meaning that patients have a systemic inflammatory response caused by cytokine storm. Since the immune system is at the center of the infection, it is crucial to regulate the dynamic balance to avoid overgrown immune responses that later result in multiple organ damage. The use of stem cells as treatment options has gained tremendous momentum over the past decade. Especially MSCs can reduce inflammatory cytokines, increase regulatory cytokines and suppress inflammation. MSCs have immunomodulatory functions and can be used in therapy to establish a balance in the immune cells of COVID-19 patients.

Introduction

Coronaviruses (CoVs) are enveloped, single-stranded RNA viruses found in the Coronaviridae family that can infect mammals and many other animals. Corona virus is a large group of viruses common among animals. They are viruses that scientists call zoonotic, meaning they can be transmitted from animals to humans. The common cold is a family of viruses that cause more serious diseases such as Middle East Respiratory Syndrome (MERS-CoV) and Severe Acute Respiratory Syndrome (SARS-CoV) [1]. The spectrum of disease caused by Coronavirus in humans can vary from simple cold to severe acute respiratory syndrome (Severe Acute Respiratory Syndrome, SARS). It can cause clinical pictures with respiratory, enteric, hepatic, nephrotic and neurological attitudes in humans and animals [2, 3]. Over the past 18 years, three highly pathogenic CoVs have emerged that can cause epidemics and fatal human diseases. Discovered in November 2020, the SARS cause was named SARS - CoV - 1 [1], and in June 2012, the Middle East respiratory syndrome coronavirus caused local outbreaks, controlled without causing a pandemic, and named MERS-CoV. SARS - CoV - 2 was identified in December 2019 after sequencing clinical specimens from a group of pneumonia patients in Wuhan, China. The disease caused by SARS - CoV - 2 was named coronavirus disease 2019 (COVID - 19). Since its first report in Wuhan, China, in December 2019, COVID-19 was declared an epidemic by the World Health Organization (WHO) on March 11, 2020, and continues to spread aggressively around the world, infecting more than 3 million confirmed cases. [4, 5]. Although COVID-19 is similar to SARS-CoV and MERS-CoV diseases, a study has shown that long-term immunity developed in people with these diseases can develop a very short-term immunity in COVID-19. While COVID-19 disrupts the distribution of immune system cells, it especially suppresses T cells that are essential for permanent immunity. As in other coronaviruses, it makes us think that a permanent immunity against COVID-19 cannot be developed due to the mutations that are formed, and that the developed immunity may be ineffective against the new mutants of the virus [6]. Many studies suggest that it is the "cytokine storm" or "cytokine release syndrome" in COVID-19 that causes acute respiratory distress syndrome (ARDS). Clinical data from severe COVID-19 patients suggest that extensive changes in serum levels of cytokines play a crucial role in the pathogenesis of COVID. Cytokine storm and associated ARDS arise with the combination of many immunoactive molecules such as interferons, interleukins, chemokines, colony stimulating factors and TNF-α [6, 7]. It is a

potentially fatal immune disease characterized by a cytokine storm, high level of activation of immune cells and the production of excessive inflammatory cytokines and chemical mediators, and is considered the main cause of disease severity and death [8].

Cytokine Storm

Cytokine storm is exaggerated with a marked expansion of multiple cytokines seen during a wide range of diseases such as infections, sepsis, organ transplantation, autoimmune diseases or drugs, immune-related treatments such as chimeric Antigen Receptor-T (CAR-T) cell therapy for certain malignancies systemic inflammatory response [9]. In severe COVID-19 patients, a marked decrease in the absolute number of circulating $CD4^+$, $CD8^+$ cells, B cells and natural killer (NK) cells was observed, while a decrease in monocytes, eosinophils, and basophils was reported. In addition, most patients with severe COVID-19 had significant increases in serum levels of proinflammatory cytokines (IL-6, IL-1β, IL-2, IL-8, IL-17, G-CSF, GMCSF, IP-10, MCP-1, CCL3 ve TNFα). In intensive care unit (ICU) cases for COVID-19 patients, the number of white blood cells, neutrophils as well as procalcitonin, C-reactive protein and other inflammatory indices is significantly higher than in non-ICUs. High cytokine level also indicates a poor prognosis in COVID-19 [2, 10]. In many studies, it has been shown that pro-inflammatory cytokines, especially IL-6, are in high concentrations in severe cases compared to moderate cases of COVID-19 [7, 11, 12]. Transcriptome sequencing has been shown to overexpress chemokines such as CXCL10 and CCL2 caused by SARS-CoV-2 infection in bronchoalveolar lavage fluid (BALF) cells from Covid-19 positive patients [13]. In addition, it has been emphasized that postmortem pathology results of lung, ARDS and T-cell overactivation in people who died from COVID-19 were caused by the increase in the number of T-helper (Th) 17 cells and high cytotoxicity of CD8 + T cells [14]. The dynamic balance provided by innate and adaptive immunity is necessary to prevent the progression of COVID-19. Plasma levels of IL-1β, IL-1Rα, IL-7, IL-8, IL-10, IFN-γ, monocyte chemoattractant peptide (MCP) -1, macrophage inflammatory protein (MIP) in patients infected with SARS-CoV-2 - 1A, MIP-1B, G-CSF, and TNF-α have been shown to be significantly higher than healthy controls. Similarly, decreases in the levels of T cells and NK cells have been observed in COVID-19 patients, which can impair the immune system [12, 15]. In a study of 452 patients with COVID-19 in Wuhan, severe COVID-19 patients had a significant reduction in total T cell count, both helper T cells and suppressor T cells. Particularly among helper T cells, a marked decrease in regulatory T cells and memory T cells was observed, while the percentage of naive T cells increased, depending on the severity of the cases. Naive and memory T cells are essential immune components, the balance of which is crucial for a highly effective defense system. Naive T cells defend against new and previously unrecognized infection with a large and tightly coordinated release of cytokines, while memory T cells mediate antigen-specific immune response. A disorder in their balance activates naive T cells compared to regulatory T cells, which contributes greatly to hyper-inflammation [12]. The innate and adaptive immune responses, activated by SARS-CoV-2 infection, lead to uncontrolled inflammatory responses, causing a cytokine storm.

Cytokine storm can result in apoptosis and vascular leakage of epithelial cells and endothelial cells, and ultimately in ARDS, other severe syndromes, and even death [16, 17]. At the same time, cytokine storm induces hypoxia, directly causing cellular damage. Multiorgan damage often occurs in COVID-19 infections [18]. Therefore, suppressing this cytokine storm may be an option in treatment. In addition to some anti-viral drugs, steroid (glucocorticoid) treatment is usually applied to patients at this stage.

Infection of cells by SARS-CoV-2

SARS-CoV-2 is a genomic, enveloped, single-stranded RNA that encodes various glycoproteins, including glycosylated (S) protein. This S-protein binds to the angiotensin I converting enzyme 2 receptor (ACE-2) in the host cell. ACE-2 receptor is highly expressed on the surface of lung alveolar type II cells (AT-2) and capillary endothelium. AT-2 cells also express type II transmembrane serine protease (TMPRSS211), which facilitates the preparation of the S-protein and thus invasion of the virus within the host cell. ACE-2 receptors are also expressed in other tissues such as kidney, liver, heart and digestive system organs; it thus explains the rapid progression to systemic inflammatory conditions as observed in critically ill patients. The fact that the thymus, bone marrow, spleen, lymph node, and macrophages do not express ACE-2 receptors means that using immunotherapeutic approaches to target the SARS-CoV-2 virus infection pathway may be feasible and provide better treatment results [19-21].

After SARS-CoV-2 enters respiratory epithelial cells, it triggers an immune response with inflammatory cytokine production accompanied by a weak interferon (IFN) response. Pro-inflammatory immune responses of pathogenic Th1 cells and mediator monocytes are mediated by suppression of membrane-bound immune receptors and signaling pathways, such as Fc and Toll-like receptors. This is followed by the infiltration of macrophages and neutrophils into lung tissue, causing a cytokine storm [22]. In particular, SARS-CoV-2 rapidly activates pathogenic Th1 cells to secrete pro-inflammatory cytokines such as granulocyte-macrophage colony stimulating factor (GM-CSF) and IL-6. GM-CSF also activates CD14 + CD16 + inflammatory monocytes to produce large amounts of IL-6, tumor necrosis factor-a (TNF-α), and other cytokines [23]. Membrane-bound immune receptors can contribute to an unbalanced inflammatory response, and weak IFN-induction can be an important enhancer of cytokine production. Neutrophil extracellular traps, which are extracellular networks released by neutrophils, can contribute to cytokine release. The cytokine storm in COVID-19 is characterized by high IL-6 and TNF-α expression [24].

IL-6 binds to sIL-6R via gp130 to form the IL-6-sIL-6R complex, which can activate STAT3 in non-immune cells. Both NF-κB and STAT3 use the IL-6 enhancer to induce various pro-inflammatory cytokines and chemokines, including vascular endothelial growth factor (VEGF), monocyte chemoattractant protein-1 (MCP-1), IL-8 and IL-6 (IL-6 Amp). IL-6 not only binds to sIL-6R to act in cis signaling, but can also bind to the membrane bound IL-6 receptor (mIL-6R) via gp130 to act in trans-signaling. It can cause cytokine storms by causing pleiotropic effects on adaptive and innate immune cells [25, 26].

Treatments for cytokine storm in COVID-19

To date, there is no specific treatment for Covid-19, but the clinical management of these patients currently includes prevention or control of infection and supportive care, including supplementary oxygen and mechanical ventilation support as needed. Prevention and mitigation of the cytokine storm could be the key to saving severe COVID-19 patients. Currently, many treatments are being evaluated in clinical trials due to the lack of high-quality evidence. Corticosteroids (Inhibit the host inflammatory response and suppress the immune response and pathogen clearance) [27], Hydroxychloroquine (HCQ) and chloroquine (CQ) (showing in vitro antiviral effects and anti-inflammatory properties) are considered potential treatments for COVID-19. CQ and HCQ can reduce CD154 expression in T cells [28] and suppress the release of IL-6 and TNF [29]. Tocilizumab (TCZ) TCZ, an IL-6 receptor (IL-6R) antagonist, can inhibit cytokine storms by blocking the IL-6 signal transduction pathway [30]. IL-1 receptor antagonists, Janus kinase (JAK) inhibitors and mesenchymal stem cells (MSCs) are currently among the treatment options.

MSCs and MSC-mediated immunomodulation mechanisms

MSCs have attracted attention because of their usefull potential, high expansion rate, and lack of ethical problems. Compared to other cells, MSCs have many advantages such as being easily obtained, being reproduced easily in a short time, and is stored for repeated therapeutic use. While MSCs express MHC-I (Major Histocompatibility Complex), they do not express MHC-II and costimulatory molecules and do not show an adverse reaction to allogeneic transplantation [31]. MSCs are defined as multipotent stem cells with the capacity to transform into multiple cells and regenerate themselves, including osteoblasts, chondrocytes, adipocytes, neuronal cells, myocytes, and β-pancreatic islet cells. MSCs are hypoimmunogenic cells and have an important role in immunoregulation. In this treatment method, which is used in some diseases and still in the trial phase; Stem cells can be obtained from many locations such as bone marrow, adipose tissue, tendons, teeth, placenta, cord blood are given to the patient-administered through intravenous [1-3].

Various studies have indicated that adult MSCs can effect on the immune T and B cell response: Adult MSCs (1) repress T cell proliferation, cytokine release, cytotoxicity, and arrange Th1 / Th2 stability [32, 34-37]; , (2) regulate the functions of regulatory T cells (Tregs) [38]; (3) While increasing B cells, it can also inhibit their proliferation and stop the cell cycle; In addition, MSCs affect the secretion of antibodies and also the production of co-stimulatory molecules of B cells [39]; (4) repress antigen presentation, activation, and maturation of dendritic cells [40, 41]; (5) they also inhibit natural killer (NK) cell activation induced by interleukin-2 (IL-2) [33]. Stem cell-derived MSCs such as embryonic stem cells (ESCs) or induced pluripotent stem cells (iPKHs) also play a regulatory role for immunomodulation by inhibiting lymphocyte proliferation and NK cells. In addition, ESC-derived MSCs inhibit the proliferation of T lymphocytes, including CD4 or CD8 T cells, and the cytotoxic effects of activated NK cells while reducing the expression of NK-activating receptors. [42, 43].

Cell-based therapies, especially stem cell therapy, have become one of the promising therapeutic approaches that provide new therapeutic opportunities for various difficult-to-treat diseases. In COVID-19 patients, a large amount of inflammatory response occurs that causes an overproduction of immune cells and cytokines, ultimately leading to a cytokine storm. In the treatment of COVID-19 patients, it is thought that MSC treatment can prevent cytokine storm release produced by the immune system and support endogenous repair with its restorative properties. [44].

The ability of MSCs to regulate inflammation depends on the functional state and location of immune cells in that area.Inflammatory signals such as C3a and C5a, which are released by damaged tissues and act as chemoattractants, cause MSCs to express chemokine receptors and migrate to that area. [45]. Generally, an active inflammatory state that can alter the immunological properties of MSCs is subject to dynamic changes. Thus, the anatomical location and inflammatory state can control the therapeutic potential of these cells in inflammatory diseases and are important determinants of the immunoregulatory properties of MSCs. While various natural immune cells such as neutrophils, mast cells, macrophages, myeloid-derived suppressor cells, dendritic cells and natural killer (NK) cells in the inflammation zone are regulated by MSCs, changes in chemokine expression can determine the functions of MSCs. For example, macrophages can exist in 2 different phenotypes, expressing their functional state, associated with different pathologies, proinflammatory (M1) or anti-inflammatory (M2). MSCs facilitate the transition from monocyte to macrophage and strengthen their pathogenic response. MSCs can also inhibit the infiltration of macrophages, monocytes, and neutrophils into sites of inflammation in a TSG 6-dependent (TNF-stimulated

gene 6 protein) mode. This mechanism is critical for the ability of MSCs to mitigate acute lung injury [4].

Inflammatory cytokines that control chemokine production cause monocytes, macrophages and neutrophils to aggregate around MSCs. Similarly, inflammation may induce the expression of IDO by MSCs and may lead to immunosuppressive effects on the migration of myeloid cells. MSCs are potent suppressors of T-cell proliferation. Various pathways are effective in the interaction between T cells and MSCs. The best described are indoleaminepyrole 2,3-dioxygenase (IDO) and prostaglandin E2 (PGE2). IDO levels increase and decrease T-cell proliferation in T cells and MSC coculture. PGE2 is formed from fatty acids through cyclooxygenase 1 and 2 (COX-1, COX-2). COX-2 expression is also increased in T-cells and MSC coculture, leading to increased PGE2 secretion and inhibition of T-cell proliferation. It reduces the secretion of PGE2, IL-2 and IFN-consisting of MSCs, reprograms macrophages to form IL-10 [5].

Immunoregulation regulated by MSCs is provided by cytokines (TGF-β1, IL-6 and IL-10), growth factors (HGF and leukemia inhibitory factor) and anti-inflammatory mediators(PGE2, TSG6, heme oxygenase 1, galectins, and extrasellular vesicles). These factors inhibit the proliferation and function of proinflammatory immune Th1 and / or Th17 cells, proinflammatory M1 macrophages, neutrophils, NK cells. By increasing the number of inflammatory immune cells such as anti-inflammatory M2 macrophages, regulatory T cells, regulatory B cells, it can further repress the activity and functions of proinflammatory cells and thus promote tissue repair and regeneration. Transplanted MSCs are attacked by complement system factors, complement activated neutrophils, and perforin positive cytotoxic cells, inducing them to apoptosis via pyroptosis. [46].

Current Studies on MSC and MSC-mediated Covid-19 Treatment

Tissue-resident MSCs may not be as effective as transplanted MSCs in restoring immunological homeostasis in damaged tissues. An advantage of in vitro-derived MSCs is that usually large amounts of cells are applied compared to baseline concentration of less than 0.05% stromal cells in the entire bone population. It has been shown that a significant percentage of MSCs are rapidly confined in the lungs after intravenous injection, and migration and retention are high in lesioned areas. When MSCs are trapped in the lungs, they exhibit the same immunomodulatory behavior as in other body parts, such as the capacity to release anti-inflammatory cytokines and antimicrobial peptides [48, 49].

Different treatments applied to Covid-19 patients so far have come to the fore. (https://clinicaltrials.gov). Some of these have focused on the use of mononuclear cells alone, the use of PRP in addition to stem cells, and the use of MSC-derived exosomes / conditioned media. One study investigated the therapeutic benefits of combining MSCs with ruxolitinib. They used MSCs as "trainers" of patients' mononuclear cells. It was transplanted with the mononuclear cells co-cultured with MSCs ex vivo. The co-culture result led to decreased expression of costimulatory molecules by T cells, increased Treg formation and TGF-β1 synthesis. Trained cells have been shown to be safe in clinical studies and exert effective anti-inflammatory effects. In addition to treatment with MSCs and different application methods, the use of products such as exosomes obtained from MSCs is also on the agenda. The source of MSCs used in the trials also formed a point of variability in studies that were determined. The most common source observed was umbilical cord / wharton gel (28 studies), followed by adipose tissue (9 out of 69), bone marrow (9 out of 69), dental pulp (3 out of 69), ECHs (2 out of 69) and olfactory mucosa, placenta, and menstrual blood (1 study each).

As a result, As treatment for COVID-19 is currently focused on supportive therapies and vaccine studies are ongoing, a large number of research studies are needed to find potential treatments to control the virus as soon as possible. The immunomodulatory and anti-inflammatory properties of MSCs in the treatment of respiratory diseases have been confirmed by 17 completed clinical studies, and more than 70 trials have been registered on this subject (https://clinicaltrials.gov). To date, 20 clinical trials have been registered on the Chinese clinical trial registry site (http://www.chictr.org.cn). Additionally, 9 clinical trials have been registered at Clinicaltrial.gov. Umbilical cord, umbilical cord blood, Wharton gel, menstrual blood, dental pulp and MSCs produced by the company are important sources of MSCs to be used in these trials. However, the process of developing new therapeutic and bringing it into clinical practice has important practical implications for the MSC treatment of COVID-19. Stem cell therapy, and especially MSCs, may be the most ideal combination of treatment to treat COVID-19 patients.

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COVID-19 IN BIOCHEMISTRY LABORATORY

Ayşe ÖZDEMİR

Introduction

2019-nCoV, or corona virus (CoV), which has high contagiousness among humans, has four types, α, β, Δ and ɣ coronaviruses, and it can cause respiratory, enteric, hepatic and neurological diseases in humans and animals (birds, fish, mammals and humans). is known to cause. It has been reported that the 229E, NL63, OC43 and HKU1 types, which were first described in the 1960s, cause mild and moderate respiratory infections in humans. In addition, severe coronavirus infections such as MERS-CoV, SARS-CoV that cause epidemics and can lead to death are also seen. The 2019-nCoV (new type of corona) virus, which emerged in Wuhan, China on December 1, 2019, was also declared as a pandemic by the World Health Organization. Corona virus, which has crown-shaped projections, is a member of an RNA virus group. 03/11/2020 Turkey has also seen the first case in history and the first death occurred on 04/17/2020. Numerical and up-to-date information on the cases can be accessed at the website of the Ministry of Health https://covid19.saglik.gov.tr and the World Health Organization's https://www.who.int/emergencies/diseases/novelcoronavirus-2019.

COVID-19 disease is transmitted by droplets, besides, it is transmitted by the contact of the droplets given by the sick individuals to the environment by coughing, sneezing with the hands of the people there and carrying them to the mucous membrane of the mouth, nose or eyes. Since viruses are detected in respiratory tract secretions, asymptomatic persons can also be contagious. Although the contagious period of COVID-19 is thought to start 1-2 days before the symptomatic period and end with the disappearance of symptoms, it is not known for sure. It is stated that viral shedding begins 1-2 days before the onset of symptoms, and that viral load can reach the highest level during the emergence of symptoms in throat swabs (also in saliva samples taken from the posterior pharynx). Although SARS-CoV-2 is rarely found in the blood, there is no information or evidence for the transmission of the virus by transfusion. The increasing number of cases and wide geographical spread of 2019-nCoV cause great concern in the world. COVID-19 disease can spread from person to person, causing more serious symptoms in the elderly and especially in people with chronic diseases. However, those with the highest risk of exposure to the disease are healthcare workers and their protection is a priority.

Naming of COVID-19 and Methods of Detection

WHO chief Tedros Adhanom Ghebreyesus opened the coding of COVID-19 on December 31, 2019 and classified a specific region or human and animal group by classifying it as "CO" for "corona", "VI" for "virus", "D" for "disease". he avoided pointing. The ICD (Classification of Diseases) codes were determined for the COVID-19 disease outbreak, which was declared an international public health emergency, and the code RA01.0 for the confirmed diagnosis of COVID-19 and the clinical diagnosis (suspected or probable) RA01.1 An urgent ICD-10 code 'U07.1 COVID-19, virus identified' is assigned to the COVID-19 disease diagnosis whose code is confirmed by laboratory tests. The first full genome of new coronaviruses (2019-nCoV) was detected in bronchial alveolar lavage fluid samples with the combination of Sanger, Illumina and Nanopore sequencing methods. With this detection, the severe infection picture caused by 2019-nCoV and the pneumonia seen were named as Novel Coronavirus Infected Pneumonia (NCIP).

Biochemistry Laboratory and Applied Rules

The rules to be done and applied regarding the admission of the patient have been determined by the Ministry of Health and have been made into a protocol. Thanks to these instructions, protection was provided and contamination during contact was prevented. A healthcare professional who helps the patient to walk within the framework of these rules, does not come into contact with the patient and his / her interests, or does not enter the patient's room is not considered risky. However, intubation, aspiration of respiratory secretions, high-flow oxygen therapy, cardiopulmonary resuscitation, dentistry applications, endoscopic procedures (bronchoscopy, videoyngoscopy, etc.), mouth-throat-nose examination, ophthalmological examinations and central catheter insertion applications have been reported to be high-risk applications for healthcare professionals. . Although the source and pathogenesis of COVID-19 are uncertain, no specific and effective treatment other than supportive care has been identified. During the increasing treatment trials, treatment options that are beneficial for some patients may not be effective in others or may show inconvenience. Although 2019-nCoV is transmitted by close contact and droplets between people and is diagnosed thanks to the feedback of many countries and the follow-up of the cases and laboratory findings in our country, the transmission time, incubation period and the duration of the virus to the external environment are not clearly known. Considering that the development of new drugs is subject to very long processes, testing previously used drugs is a promising approach in terms of contributing to the treatment. In addition, it will not be possible to make statistics on this subject before the disease is eliminated or disease spread is prevented. The World Health Organization (WHO), "and that they were in cautious optimism about Turkey began to stabilize and the number of cases in the last week (April 23, 2020) the rate of increase of about 47 percent of cases around that" reported. It is also seen in the statement of the WHO that it would be appropriate to make an important evaluation and compare the results with the previous week instead of statistically.

COVID-19 Diagnostic Methods and Biochemical Tests

Among the diagnostic methods of COVID-19, various immunological tests with nasopharyngeal swab and Quantitative Real Time Polymerase Chain Reaction (qRT-PCR) method is performed by targeting specific genes. COVID-19 is detected in our country with Diagnostic Laboratories Authorized by the Ministry of Health. Since more than one test has to be done, the number of tests available does not represent the number of people tested, so the total number of tests can be seen in large numbers.

Although there is no standard serological test to date, it has been reported that it would be appropriate to keep acute and convective serum samples with the consent of the patient for epidemiological evaluation. It is recommended to take nasopharyngeal and oropharyngeal swabs or washing samples together, especially in outpatients, since the virus is highly reproduced in upper respiratory tract tissues. From symptomatic cases, nasopharyngeal and oropharyngeal swab, nasopharyngeal aspirate or nasal washing, sputum, bronchoalveolar lavage, (endo) tracheal aspirate, biopsy or autopsy material and serum (two samples taken 2-4 weeks after the acute and acute phase), whole blood and urine samples It is stipulated to be used in diagnosis by the Ministry of Health. Respiratory samples taken are evaluated in laboratories microbiologically for SARS-CoV-2.

COVID-19 and rRT-PCR (real-time reverse transcription polymerase chain reaction)

Many analyzes have been developed that detect the COVID-19 virus and are still being developed. For diagnosis in routine and reference laboratories, virus-specific ORF 1b or PCR (real-time reverse transcription polymerase chain reaction) method, which targets the

nucleoprotein gene, is used. Compared with traditional PCR analysis, diagnostic analysis based on real-time PCR technology has increased speed and dynamic range; It is also a method that enables quantitative analysis of gene copies and has the potential for increased specificity when using nucleic acid probes. Optimized real-time PCR studies can be highly sensitive in a nucleic acid sample, detecting even a small amount (1-10 copies) of the target gene with low copy number. In real time PCR applications, more cleanliness and precision are required in the laboratory to prevent contamination. Here, Step 1 is to carefully perform and optimize the nucleic acid isolation using one of the many methods available in an environment free of PCR inhibitors. In the 2nd step, it is necessary to convert mRNA to cDNA. The purpose of this is to examine mRNA more easily and in detail thanks to the more durable cDNA and to determine the expression level of genes in the cell being examined. Reverse transcription of RNA adds an additional variable that can affect quantitative data. For the test itself, different options have been developed for real-time detection of products, including dye-based tests, hydrolysis probes, and hybridization probes. Each of these options has benefits and drawbacks.

Using PCR, specific sequences in a DNA or cDNA can be copied or amplified millions of times using sequence-specific oligonucleotides, heat stable DNA polymerase, and thermal cycling. In real-time quantitative PCR, the PCR product is measured at each cycle. By monitoring the reactions during the reaction's base amplification phase, users can determine the initial amount of the target with great precision. By monitoring the reactions during the exponential amplification phase of the reaction, the initial amount of target can be determined with great precision. PCR theoretically replicates DNA exponentially and doubles the number of target molecules per amplification cycle. In real-time PCR, the amount of DNA is measured after each cycle by means of fluorescent dyes that give an increasing fluorescent signal in direct proportion to the number of PCR product molecules (amplicons) produced. The data collected in the exponential phase of the reaction gives quantitative information about the initial amount of the amplification target. Fluorescent rapoters used in real-time PCR include double-stranded DNA (dsDNA) binding dyes or dye molecules linked to PCR primers or probes that hybridize with the PCR product during amplification.

The change in fluorescence during the reaction is measured with a device that combines thermal cycling with the ability to scan fluorescent dye. The real-time PCR instrument plots the fluorescent irradiance against the number of cycles, generating an amplification graph representing the product accumulation during the entire PCR reaction.

The advantages of real-time PCR include:

• Ability to monitor the progress of the PCR reaction in real time

• Ability to precisely measure the amount of amplicon in each cycle, enabling extremely accurate measurement of the amount of starting material in samples

• Amplification and detection takes place in a single tube and eliminates post-PCR manipulations.

COVID-19 Patient Assessment with rRT-PCR

If a negative result is obtained from a patient with high suspicion of COVID-19 infection using rRT-PCR and only upper respiratory tract samples were collected, additional samples containing lower respiratory tract samples should be taken from the patient and rerun. Negative results may occur due to sampling at a very early or late stage of infection, poor quality sample with too little patient material, not properly sent, or technical reasons such as PCR inhibition or virus mutation. Even if a negative result is one or more than one, the possibility of COVID-19 virus infection cannot be excluded. The uncomplicated disease picture is evaluated and it is

decided that those with positive test results and those with negative results will continue to be followed at home or taken to the hospital. In patients with mild pneumonia findings, it is decided according to the need for supportive treatment with the clinical picture of the patient and whether the patient will be able to isolate himself at home, and whether he will be distressed in terms of coordination, in order to decide whether the test result is positive and negative at home. Although viral RNA was detected negative in two consecutive respiratory tract samples, it was observed that it could become positive again later due to methodological reasons. Even if different respiratory pathogens are detected, all patient samples that meet the possible case definition should be evaluated for SARS-CoV-2, considering that the patient may have COVID-19 infection at the same time. In cases with severe pneumonia, whose blood lymphocyte count is 40 mg / l or ferritin> 500ng / ml or D-Dimer> 1000 ng / ml as a poor prognostic criterion in blood tests, treatment is started without waiting for the test result. In addition, those with positive test results who completed the recommended treatment period and whose symptoms and findings improved are discharged; Patients whose symptoms and signs continue or whose clinical condition worsens continue to be controlled with new treatment options in the intensive care unit. Patients with troponin elevation and arrhythmia, and those with a high Lactate> 2 mmol need to be evaluated in terms of need for intensive care. PCR samples are taken again 24 hours after the test result is found to be negative. While those who are found to be negative for the second PCR sample are evaluated in terms of alternative diagnoses, those with a positive second PCR sample continue with COVID-19 treatment. Technical reasons such as too little patient sample in the infected individual, taking samples at a very early or late stage of the infection, not sending it properly, PCR inhibition, and factors indicating the wavy scattering of the SARS-CoV-2 virus in symptomatic and asymptomatic cases may cause a negative result. To understand the evolution and epidemiology of the virus; To be able to analyze the epidemic by looking at the spread from a certain source, the time of initiation, the transmission routes, and the ways of spread in the host and nature; Sequence data are also important for predicting differences between different countries and continents on the spread of the virus and to be able to provide the most effective action strategies.

Biochemical Tests and Serological Diagnostic Tests in the diagnosis of COVID-19

The contributions of the Biochemistry department are important for the diagnosis of COVID-19 disease and patient follow-up. Because, starting with patient classification, biochemistry parameters are used in the evaluation of the prognosis of the patient and in the follow-up of the treatment. The predicted blood tests are as follows:

Complete blood count	Urea, creatinine
D-dimer, ferritin, troponin	Glucose
Total bilirubin	AST, ALT
LDH, CPK	Sodium, potassium, chlorine
C-reactive protein values	

With the start of emergency pandemic outpatient and pandemic clinics, the test profile coming to the Biochemistry laboratory has also changed. In addition to the tests mentioned above, the demand for parameters including inflammation indicators, blood gas analysis, hematological parameters, control of the coagulation system and parameters reflecting organ functions has increased. Because, according to the General Directorate of Public Health Directive, hemogram, CRP (C-Reactive Protein), LDH, D-dimer, ferritin, blood gas tests were requested from each patient who applied for an emergency pandemic with COVID-19 pre-diagnosis and other complaints, CT findings, clinic and this Biochemical parameters were

evaluated together and the decision was made for pandemic hospitalization and outpatient treatment. Although retrospective studies on these tests are thought to be better evaluated, low saturations, increased levels of LDH (lactate dehydrogenase) enzyme, D-dimer increases due to impaired coagulation system and intravascular clot formation, changes in CRP levels and decreased lymphocyte values have started to be detected frequently. It is thought that low serum albumin levels in COVID-19 patients may be associated with an increased risk of death. In addition, the increase in the neutrophil / lymphocyte ratio (NLR) is thought to be an important marker in showing the severity of the disease. Glucose is especially important for follow-up in COVID-19 patients with diabetes.

Serological diagnostic tests are used in the diagnosis of COVID-19, including ELISA or rapid antibody tests that detect IgM / IgG based on bedside chromatographic tests and immunoassay methods. The COVID-19 immunochromatographic test is a lateral flow immunoassay. Lateral flow immunoassay qualitatively evaluates the presence of an analyte from a patient sample or sample. Detected analytes are IgG and IgM antibodies specific for SARS-CoV-2. Ig M and Ig A become positive 3-6 days after the onset of symptoms, and Ig G becomes positive 10-18 days after the onset of symptoms. However, none of them had a positive predictivity rate of 100%. Membrane-based and automated immune methods are used for the detection of these antibodies, but both have advantages and disadvantages compared to each other. However, serological tests allow the attack rate and the severity of the outbreak to be evaluated retrospectively.

In foreign-sourced publications, the typical symptoms of 2019-nCoV infection are fever, lymphopenia, cough, myalgia or fatigue, hemoptysis, acute cardiac injury, pneumonia; It has been reported that a decrease in the number of CD4 + and CD8 + T cells and lymphopenia is higher than in leukopenia, and higher levels of IL-1beta and other inflammatory cytokines are found in plasma. In children, it is recommended to measure interleukin-6 (IL-6) levels. It is thought that the increase of the LDH enzyme may be a biomarker that may indicate lung damage in patients with severe COVID-19. It has been found that the high levels of CRP are associated with the severity of the COVID-19 disease. Although plasma sodium, potassium and calcium concentrations are observed to be low in COVID-19 patients, its relationship with the course of the disease has not yet been clarified. It has been reported that prothrombin time and D-dimer levels may also be directly proportional to the severity of the disease. It is stated that cardiac biomarkers such as Cardiac Troponins (TnT / TnI), BNP / NT-proBNP (B-type Natriuretic Peptide) and CK / CK-MB (Creatine Kinase) are biochemical tests that should be followed in terms of the severity of the infection and prognosis. Patients determined according to the criteria in the guideline issued jointly with the Scientific Committee of the Ministry of Health should be monitored in the intensive care unit upon the development of poor prognosis criteria (blood lymphocyte count 40 mg / l or ferritin> 500ng / ml or D-Dimer> 1000 ng / ml as a poor prognostic criterion in blood tests). It is recommended to start. During COVID-19 infection, with or without sepsis and ARDS (Acute Respiratory Distress Syndrome) findings, macrophage activation syndrome (MAS) picture characterized by cytokine storm due to hyperinflammatory response or acquired (secondary) hemophagocytic lymphohistiocytosis (sHLH) findings It is stated that liver enzymes may increase secondary to cytokine storm and drugs used, therefore the evaluation of clinical and laboratory findings should be done very frequently. In addition, along with the prolongation of hospital stay and treatment protocols, an increase in total biluribine amounts and ALT (Alanine aminotransferase), AST (Aspartate aminotransferase) and GGT (gamma-glutamyl transferase) levels were detected. Although SARS-CoV-2 is rarely found in the blood, there is no information or evidence for the transmission of the virus by transfusion.

Laboratory Safety and Risk Assessment

All procedures in the laboratory process, based on risk assessment and in accordance with the relevant protocols, should be carried out by trained and conscious personnel. Considering that every sample sent for this purpose may belong to a patient with COVID-19, our laboratory functioning has undergone some changes with COVID. In the realization of this, the guide created by WHO for laboratory analysis of suspected cases for coronavirus disease published on March 19 and the "COVID 19 Laboratory Biosafety Guide" published by the Ministry of Health General Directorate of Health Services on April 8 were used. In order to protect employee safety, personal user equipment (gowns, masks, goggles, face shields, surgical caps and gloves) has been completed and its use has been given importance. Cleaning was carried out by using 70% ethyl alcohol and bleach (with different dilutions for general surface cleaning and spills as specified in the guide) and the frequency of in-laboratory cleaning was increased. The number of hand disinfectants has been increased in every corner and in the analysis section. Manual analysis of stool, urine and body fluid samples were made in the safety cabinet by increasing measures in the storage and disposal of samples (Figure 1).

Figure 1. Biochemistry Laboratory Work in the COVID-19 Process

In addition, the in-lab working procedure and approach to analytical process were reviewed and our technicians were planned to work in accordance with working hours. It is stated in the guide that "it should be ensured that the air flow systems used in laboratory ventilation do not endanger the safe working environment". By making regulations on air flow in accordance with this procedure, the risk of COVID-19 transmission of healthcare workers working in the laboratory was tried to be minimized. Measures to be taken in Medical Biochemistry Laboratories during the COVID-19 process have been evaluated as preanalytical, analytical and postanalytical. In the preanalytical process, samples used in Medical Biochemistry Laboratory tests consist of blood, urine, saliva, stool and various body fluids. In the World Health Organization's guide titled "Laboratory biosafety guidance related to coronavirus disease 2019 (COVID19)", "Patient samples whose positivity is suspected or confirmed as UN3373 -" Biological Substance Category B "; Viral cultures or isolates Category A, UN2814 - “must be transported as an infectious substance, affecting humans”. According to the obtained and uncertain information, the presence of virus in urine and blood is absent or much less than other biological materials. Considering this information, samples were transported separately from other samples with suitable transport containers instead of vacuum systems and the laboratory unit was checked for infection and warned. Samples that need to be sent outside the institution, even samples that are not diagnosed with COVID-19, are transported in accordance with

standard transport procedures due to the potential infectious risk. During the sampling and sampling stages, joint local risk assessment is carried out with the microbiology laboratory. The risk of aerosol formation is taken into consideration in the use of centrifuge. Laboratory planning has been made in the analytical process to minimize the risk of possible exposure from spills or aerosols. Since PCR has not been implemented yet, suspicious clinical samples coming to the laboratory are kept separate from other samples. The post-analytical process applications were given to the staff in the form of training.

Result

As can be seen, laboratory tests make an important contribution to the monitoring of the diagnosis and treatment of COVID-19. However, laboratory evaluation of children in this infection, which seems to be an adult disease, has not been clarified. All laboratory professionals have made extraordinary efforts in managing this crisis, gaining new experiences in managing resources in health centers. The importance of multidisciplinary work compliance during the COVID-19 outbreak has once again been demonstrated.

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SERVICE DELIVERY IN AUTHORIZED DIAGNOSIS LABORATORIES for COVID-19

Ayşegül ÇOPUR ÇİÇEK

In the process where the number of cases associated with COVID-19, which was first seen in Wuhan, China in December 2019, increased significantly in a short time and turned into a pandemic, the Ministry of Health (General Directorate of Public Health) sent official letters to the governorships of 81 provinces regarding the authorization of a new type of coronavirus (COVID-19) diagnostic laboratory. Primarily 37 laboratories were first authorized from 21.03.2020. While COVID-19 continues to spread in Turkey, The service in laboratories where COVID-19-related infections are diagnosed has started to be carried out under the coordination of the General Directorate of Public Health (GDPH) Microbiology Reference Laboratory and the Department of Biological Products, and the process has still continued in this way. Currently, 274 public and private laboratories are authorized. Public, university hospitals and private laboratories that are not officially authorized by GDPH are not authorized to perform any tests for the diagnosis of COVID-19. COVID-19 authorization protocols have been signed with the relevant laboratories. In the signed protocol; Many issues such as physical space and other infrastructure, manpower, biosecurity, recording, archive, confidentiality have been taken into account in laboratories that have received a license for the microbiology laboratory. In COVID-19 authorized diagnostic laboratories, as in all other test processes, pre-preanalytical, preanalytical, analytical, postanalytical and post-postanalytical processes are followed and studied.

PRE-PREANALYTIC AND PRE-ANALYTICAL PROCESS

- Test request,
- Training of staff for sampling,
- Taking the sample by the appropriate method,
- Taking the sample at the right time,
- Taking the sample into the correct transport environment,
- Closing the covers of sampled types tightly,
- Recording the sampling time,
- Correct and adequate labeling on sampled tubes. Taking all measures for patient safety (such as identity verification),
- Proper storage of samples,
- Transport of samples,
- Sample acceptance,
- The process is maintained in stages such as the preparation of samples for analysis.

As stated in the guide prepared by the Scientific Committee, samples must come to the authorized laboratory with below conditions;

- Barcoded sample printed by the hospital information management system (HIMS),
- 2019 New Coronavirus Infection (COVID-19) Case Information Form and
- "Laboratory Request Form" that can be obtained from the Public Health Management System (PHMS) with a barcode.
- Also, in the COVID-19 diagnostic laboratory, referral and admission to the Laboratory Information Management System (LIMS) are made.

These systems contain the following information:

- Patient ID Information
- Case type (outpatient, service…)
- Clinical signs and symptoms
- Risk factors
- Epidemiological data
- Laboratory order data (combined, tracheal aspirate)
- Physician, institution information
- Additional information (such as smoking inquiry in PHMS)

- During the COVID-19 PCR test request, the requested information about the patient must be filled in completely in both the Case Report Forms and PHMS and LIMS entries in a way to ensure the patient, employee, and information security. At this point, laboratory experts, clinicians, external stakeholders, and Provincial Health Directorates should use good communication strategies professionally required by teamwork. In addition, there is a need for improvements to make all transactions with simpler, faster, and more effective applications, especially the integration of the information automation systems of the institutions to PHMS and LIMS.

In addition, all samples taken should be considered potentially infectious, sampling should be considered as the process that causes aerosolization, and not only respiratory samples, but also all samples should be taken by the trained personnel with strict adherence to all established biosafety and procedures without risking patient and worker safety.

Acceptance and rejection criteria of the samples taken should be determined.

Reasons for Rejection of COVID-19 PCR Sample

- Deficiencies in records and forms,
- Barcode unsuitabilities of the sample,
- Insufficient sampling,
- Viral Transport Medium (VTM) with the passed expiration date,
- Storage of the sample in unsuitable conditions,
- Opened, spilled, leaked sample,
- No swab in the viral transport tube,
- The presence of blood or other inhibitors in the viral transport tube that could affect the PCR reaction.

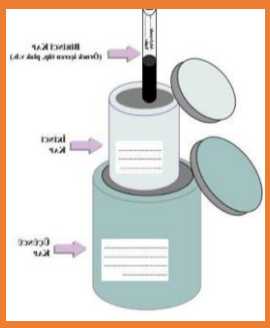
The kits for the COVID-19 PCR test are validated for respiratory samples, and the samples taken are respiratory nasopharyngeal swab, nose, throat, tracheal aspirate, bronchoalveolar lavage. The procedure for samples other than respiratory samples can be established.

Storage of samples / Biosecurity

- It can be kept at 2-8 °C for up to 72 hours at the latest.
- If it will wait longer, it should be kept in freezers at -80 °C.

As stated in the Covid-19 Diagnostic Laboratories Biosafety Guide of the Ministry of Health;

- 3.1.12. Samples from suspected or definite cases must be transported as UN3373 (the biological substance in category B).
- Viral cultures or isolates should be transported in category A, UN2814 (as human infectious agent). If an internal sample transfer is required, a three-container transport system should be used.



ANALYTICAL PROCESS;

- Control of materials and other equipment (vortex etc.)
- Reagent and kits
- Devices (Rotorgene-BioRad, Roche etc.)
- Disposable consumables (e.g. pipette, pipette tip, gloves, plastic pipettes)
- Biosafety cabinet, PCR cabinet
- It is a process where many variables such as well-trained, competent personnel with PCR experience are together and that tests should be studied meticulously under the coordination of a microbiologist. According to the application technique of the kit, most of the stages are a labor-intensive testing process that requires manual work. PCR test analysis for COVID-19 should not be understood as a test where the technician works by putting the sample into the device. One of the most important aspects of this process is that GDPH distributes the domestic and national kit (changed 6 versions in 6 months) produced by Bioeksen company to authorized diagnostic laboratories. For the last 1 month, the supply of local and national kits, such as Gensutek and RTA, has been provided by the State Supply Office. Quality control studies are carried out with the MOTAKK external quality control program.

POSTANALYTIC AND POST-POSTANALYTIC PROCESS

- Interpretation and Reporting of Results,
- Sample Storage and Obtaining of Reference Sample,
- Recording / Archiving
- Panic / Critical Value Statement

Interpretation and Reporting of Results

Interpretation and reporting of COVID-19 test results require microbiology expertise when it takes into consideration varying kit versions, protocols, and devices. Therefore, the interpretation of the results is a very critical stage for COVID-19 tests, as in all other tests. Test results should be interpreted by giving information as follows by performing the duty of consultant by the microbiologist;

- should be evaluated together with the clinical findings of the patient,
- If the result positive, the presence of SARS-CoV-2 RNA in the patient sample should be shown,
- If the result negative, it should be shown that SARS-CoV-2 RNA is not detected in the clinical sample and it will not exclude the diagnosis of COVID-19,
- If the suspicion of COVID-19 continues, the test should be repeated with a new sample after 24 hours.
- The day after the onset of symptoms or contact, the sampling and sample quality will affect the result,
- Again, it is preferred that the sample belongs to the lower respiratory tract if possible.

Results should be evaluated with a holistic approach, not only with clinical information but also taking into account all processes of the test. Improvements should be made for false negativity, false positivity and test repetitions.

In cases where definite results cannot be given; samples should be sent to the National Influenza Center and Respiratory Viruses Reference Laboratory for further evaluation.

• Since the results cannot be entered into the information management systems of the institutions, accessibility for results notifications via PHMS and LIMS should be ensured in order for the clinician and the patient to reach the results.

•Not only in delivering results to the patient; the delivery of the test result approved by the microbiologist to the provincial health directorate officials and field workers in the form of a regular data flow is also of key importance in the control with the epidemic.

• LIMS registration should be made on the same working day when the test results studies are completed and the test results should be sent to the e-mail address determined by the Ministry of Health as an excel file by the Medical Microbiology Specialist in charge of the COVID-19 Diagnostic Laboratory.

• The laboratory supervisor must send the device quality control records, the number of tests studied and the number of repeated tests to the Reference Laboratory until the end of work on Fridays.

<u>Panic / Critical Value Statement</u>

- Panic value declaration is NOT made over LIMS.
- One-on-one communication with the patient's physician can be made / SMS can be sent.
- Provincial Health Directorates follow the results instantly.

- Persons authorized through LIMS / PHMS can see it.
- Results from LIMS are automatically transferred to e-Nabız.

Sample Storage and Reference Sample Submission

At the beginning of the process, the General Directorate of Public Health was asked to send negative and positive samples every week from the laboratories authorized to study COVID-19 diagnostic tests. However, as of 21.07.2020, with the official letter from GDPH, it was shared with the field that negative samples should be destroyed according to biosafety rules and positive samples should be stored within laboratory facilities. Reference samples should be recorded retrospectively and stored in deep freezers.

Confidentiality and Privacy

In accordance with the contract made with authorized laboratories, the confidentiality of the information and documents regarding the tests and their results within the scope of COVID-19 should be preserved and these information and documents should not be shared with any institution or organization, including other units of its institution, except the General Directorate of Public Health.

Recording / Archiving

It is told that the section titled " Duties and Responsibilities for the Functioning of the COVID-19 Diagnostic Laboratory to be authorized " in the " o " clause " All studies records, sample records, and result forms should be kept indefinitely, and calibration and control records should be kept for 10 (ten) years and It should be kept in an environment that only responsible people can reach."

With the intensive and devoted work of Microbiologists in COVID-19 Diagnostic Laboratories, which serve 24/7, approximately 10 million tests have been studied as of now.

ENCEPHALITOZOONOSIS IN LABORATORY RABBITS AND SCIENTİFIC STUDIES IN TURKEY

Banu Çiçek YÜCESAN

Since rabbits are suitable role models for a variety of human diseases, they are often used in laboratories. Most laboratory rabbits are housed in modern conditions that prevent exposure to common parasites. Rabbits reared in laboratories are generally commercially grown and therefore protected from parasites. However, laboratory rabbits that encounter rabbits purchased from outdoor units or leaving the wild environment may become infected and contaminate feed and bedding materials in their areas. Thus, there may be parasites in animal production facilities.

Encephalitozoon cuniculi is a protozoan infection that can infect many domestic mammals, including rabbits, mice, rats, hamsters, guinea pigs, dogs foxes, cats, horses, pigs, squirrels, monkeys, and humans. *E cuniculi* is an obligate intracellular parasite whose spores are highly resistant, including the phylum Microsporidia. Microsporidia receive little attention in the veterinary medicine curriculum. Because they are only considered important as rabbit and fish parasites. However, Microsporidia are also important parasites in humans, and some species cause serious illness and death in immunocompromised individuals. E. cuniculi also occurs in the wild, and Encephalytozoonosis is one of the most common health problems in rabbits and is found in industrial and family farms and pet, zoo, and laboratory rabbits in many countries (1)

The genome of *E. cuniculi* has 2.9 million base pairs. With this structure, it is the smallest protozoon in eukaryotes. Microsporidia have many features in common with prokaryotes. This parasite does not have mitochondria, centriol and peroxisome, and its golgi apparatus is atypical and its ribosomes are similar to them (2). Its spores are oval and thick-walled and measure are 1.5 µ - 2 µ to 2.5 µ - 4 µ. Spores of *E. cuniculi* have a coiled polar filament. It causes degradation of ether, hydrogen peroxide or heat filaments (3).

The life cycle of *E. cuniculi* is 3 to 5 weeks. It is passed on to the hosts through ingestion and inhalation of spores. In addition, it becomes infected with transplacental transmission (4).

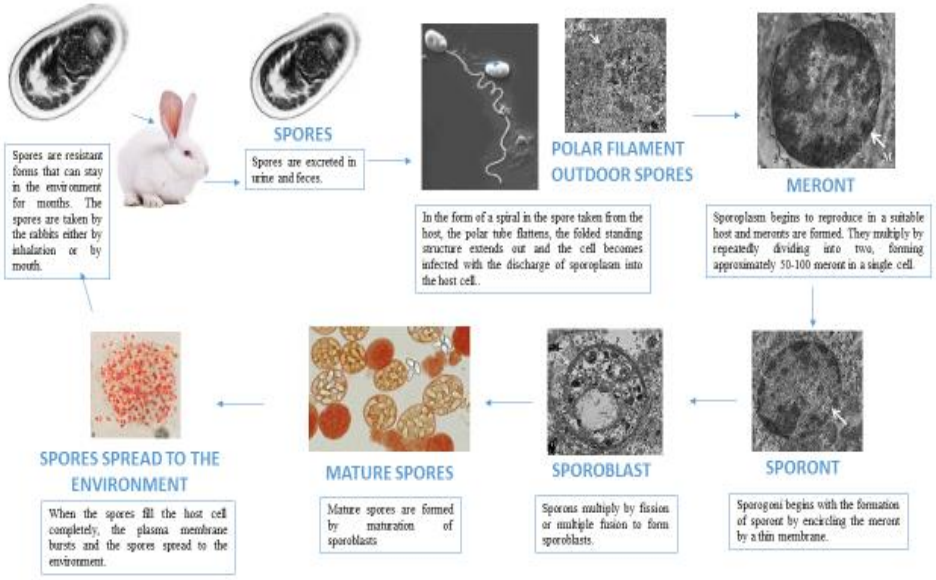


Figure 1: *E. cuniculi* life cycle

Spores can penetrate a host cell through its polar tubes. The sporoplasm is divided into two and turns into the proliferative stage, meront. Meronts can undergo several replications before turning into sporont. Sporonts turn into sporoblasts and they turn into spores. When the host cell is filled with mature spores, the cell bursts and the spores are released (5) (Figure 1). These spores are resistant to environmental factors and can survive for several years. Spores pass into the rabbit's urine, begin to be excreted approximately 35 days after infection, and continue to be excreted for 2 to 3 months (2). The infectious dose required to cause disease in 50% of rabbits is only 46 *E. cuniculi* spore. The primary route of transmission of *E. cuniculi* among rabbits is through the ingestion of spores shed in the urine. It is unclear whether the organisms undergo first proliferation in intestinal epithelial cells. However, it is thought that it invades the intestinal epithelium (6). Because organisms soon appear in many tissues of the rabbit. During the first 30 days, it is most commonly found in the kidneys, liver and lungs. At this point, rabbits are usually asymptomatic and damage to organs is limited. It is then found mostly in the kidneys, brain, eyes and heart (7). The parasite can remain in these organs indefinitely without causing clinical symptoms in the animal. However, some rabbits are more affected by invasion of these organs and clinical diseases may develop. Rabbits younger than 6 weeks old are more likely to develop severe illness (8). Nonpurulent-granulomatous lesions are characteristic in the brain and / or kidneys (9). Transplacental transmission of *E. cuniculi* has been detected in rabbits, mice, dogs, foxes, horses and guinea pigs (7). The main organs of rabbits that are mainly affected by the parasite are the kidneys and brain. Antibodies develop three to four weeks after infection in parallel with antibody titers that increase over time, normally the first changes occur in the kidney and then in the brain. The pathogen is mainly localized in the renal cortical tubular epithelium and sometimes in the glomerular epithelium (1).

Although E. cuniculi is subclinical in rabbits, the symptoms are usually neurological when seen clinically. It includes ataxia, tremors, paresis, hyperesthesia, opistotonosis, torticollis, convulsions and paralysis (7).

There are three main genotypes or strains of *E. cuniculi*. These are *E. cuniculi* genotype 1 or rabbit strain, genotype 2 or mouse strain and genotype 3 or dog strain (10). Since *E. cuniculi* was first identified as an infectious agent in rabbits in 1922, its genotype isolated from rabbits in later years was named genotype I. It was first reported as an important cause of neurological disease in laboratory rabbits in 1922 (10). Later, Cox et al. demonstrated the life cycle and pathogenesis of this obligate intracellular parasite in experimental and naturally infected rabbits (8).

Encephalitozoonosis in rabbits is easy to diagnose after death. Because its lesions are characteristic. *E. cuniculi* can be identified by staining with Gram, Giemsa and Goodpasture carbol fuccin. Differential diagnosis with *Toxoplasma gondii* should be made by staining methods. Serological methods are also used to detect *E. cuniculi.* These are IFA (Indirect Fluorescent Antibody), ELISA (Enzyme Immune Assay Test), complement fixation, immuno peroxidase, carbon immunization and indirect microagglutination methods. Also, rarely, skin bundles, mouse inoculations and cell cultures can be used for identification. Polymerase chain reaction (PCR) is a specific test used in the detection of *E. cuniculi* as in many parasites today.

Although there is not much prevalence data for *E. cuniculi* in rabbits in the United States, serological studies in rabbits in Europe and Africa show that it is common (2).

E. cuniculi is an opportunistic pathogen for humans. It can occur especially in diseases such as immunocompromised HIV / AIDS, organ transplantation or CD4 + T lymphocyte deficiency. This parasite is much more common in animals than humans and therefore it is a

rare disease of zoonotic character in humans. Studies have also shown that human-to-human transmission is possible through the transplant of an infected organ from an infected donor (11).

Encephalitozoon cuniculi studies in Turkey

The studies ever conducted in Turkey are the works that are done on rabbits and *E. cuniculi*'s disease in humans.

E. cuniculi work for the first time in Turkey in 1983 was carried out in the Ankara region. In this study, the disease was found incidentally in 4 rabbits and was recorded as the first detection (12).

Eröksüz H et al (1999), in a study they conducted in a colony of 40 rabbits, found infection in 92.5% macroscopically and 100% microscopically (9).

In the study conducted by Eröksüz Y et al in Elazığ region, it was observed that 65.3% of 150 rabbits were seropositive (13).

Carhan et al (2015), they detected for the first time *E. cuniculi* that an animal care workers in Turkey and published (14).

Özkan et al (2018), have carried out the first year of the rabbit eye in the *E. cuniculi* molecular assay and genotyping in Turkey (15).

Özkan et al. (2019), investigated that the relationship between *E. cuniculi* seropositivity and renal function markers in clinically healthy rabbits could be useful in the diagnosis of the disease and found these two parameters to be related (16).

Rabbits are widely used as a model in many in vivo studies, including biomedical and surgical studies, atherosclerosis research, antibody production. Although most of the latent *E. cuniculi* infections do not cause clinical symptoms, the infections can have a significant impact on the results of in vivo studies. Therefore, it is necessary to take serious precautions against *E. cuniculi* infection in laboratory rabbits raised for scientific studies. It is also known that microsporidia are important opportunistic pathogens that affect immunocompromised individuals. Therefore, any situation that may cause immunosuppressed patients to encounter *E. cuniculi* in their working environments should be eliminated. It should not be overlooked that this issue may be a public health problem.

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CANCER AND PHYTHOTHERAPY

Funda KARABAĞ ÇOBAN

Summary;

Cancer is one of the most important diseases, especially after cardiovascular diseases, which are common worldwide. Many studies are conducted for the treatment of cancer types whose causes and types are very different from each other. Due to the side effects of drugs used in cancer treatment, there are numerous studies that researchers have conducted using extracts or active ingredients of plants found in nature on different cancer cells or in experimental animal models, which can prevent or reduce the side effects of these drugs and the damage they give to cells. This review presented is based on plants and their active ingredients that are thought to have bioavailability against cancer.

Key Words: Cancer, antioxidants, plants

introduction

Plants have been the subject of research for many years as they are an important resource for the development of new drugs and for researchers. In general, when looking at the herbs that are considered to be used for cancer treatments, it is seen that they have a long history and that they have been used as therapeutic in such diseases in the past. Therefore, plants are the primary source for the production of drugs to be used in the treatment of cancer (1). Phytotherapy, which has become more popular in recent years, is applied in cancer treatment as well as in different diseases.

Phytotherapy, In order to protect from diseases and support treatment, plants with scientifically proven medicinal effects, their parts carrying their active ingredients and / or their natural products obtained through a process, and standardized pharmaceutical forms (tablets, capsules, tinctures ...) and herbal medicinal products application using products. Chemical substances synthesized by plants constitute the basis of herbal treatment. These chemicals cause a number of physiological changes in the body and are useful in curing some diseases (2).

Studies show that cancer is directly related to diet. It has also been agreed that many cancer risks are related to changes in dietary compounds. People with a much higher intake of antioxidants through fruits and vegetables have been found to have a much lower cancer rate than other people (3,4).

When the literature is examined; There are many studies on the presence of oxidative stress in the pathogenesis of cancer (5). Therefore, antioxidant supplements have come to the fore against the presence of reactive oxygen that causes oxidative stress. In fact, there are profound disagreements about whether oxidative stress causes cancer or suppresses it. (6).

Cancer cells actually produce much more reactive oxygen than abnormal cells. This may be because their mitochondria are defective. (7). In addition, the amount of ROS production has been associated with the progression and spread of cancer (7). For this reason, researchers have turned to antioxidant substances found in plants and continue their research on whether they can be a therapeutic agent in cancer.

Effect of Antioxidant Compounds on Cancer

Anti-oxidants are known to have an effect against the negative situation caused by ROS (10,11), especially herbs with antioxidant effects (8,9). Various clinical and experimental studies have shown their effects, especially in the prevention and treatment of life-threatening diseases

(10,12). Most medicinal herbs with antioxidant activity show promising results in cancer treatment. (13,14,12,5). Promising results have been obtained in preclinical cancer prevention studies or in autoimmune system stimulation. (15,16). However, it is currently unclear whether dietary antioxidants will reduce the risk of developing or prevent cancer.

Compounds with Anticancer Properties in Plants

Vegetables, fruits, nuts and grains are major sources for the maintenance of human health due to the contents of fibers, trace elements, essential oils and vitamins, and for the prevention of cancer due to their effects on different cellular mechanisms. (25,26,27,28). Plants have also observed a wide range of cancer incidence lowering effects such as flavonoids monophenols and polyphenols terpenoids and nitrogen containing alkaloids.

When we search the literature on this subject, we can see that the active ingredient or plant extracts containing numerous antioxidants are used in different cell culture lines or animal models. It has been observed that these substances are generally alkaloid, mostly phenolic and terpenoid structures.

1. Alkaloids

Alkaloids normally constitute a fairly numerous organization of compounds containing cyclic systems having at the least one simple nitrogen atom. Today, many alkaloids are used withinside the pharmaceutical industry. These compounds have a huge distribution withinside the plant country and are in large part located in flora belonging to the households Leguminosae, Menispermaceae, Ranunculaceae, Loganiaceae and Papaveraceae (17,18,19). Various alkaloids which include camptothecin, vincristine, vinblastine, berberine, sanguinarine, evodiamine, piperine, matrine, and tetrandrin are properly referred to as amazing chemotherapeutic agents.

2. Phenols

Recent findings factor to numerous phytochemicals, which include phenolics, in those anticancer properties. Both monophenolic and polyphenolic compounds from a huge style of natural foods, spices and drinks had been proven to inhibit or attenuate the onset, progression, and unfold of cancers in vitro and in vivo. They alter those anticancer results of phenolics via way of means of modulating cell mechanisms and regulating boom elements and receptor interactions, and via way of means of regulating the cellular cycle, which include kinases and transcription elements, and making sure cellular survival (20). An vital attention is at the inhibitory results of phenolics at the stress-activated NF-κB and AP-1 sign cascades in most cancers cells, which might be appeared as key healing targets. Phenolics can beef up the body's immune gadget to apprehend and smash most cancers cells and inhibit the improvement of recent blood vessels (angiogenesis) crucial for tumor boom. They additionally lessen the adhesion and invasiveness of most cancers cells, hence decreasing their metastatic capacity (20). Plant phenolics seem to have each preventive and healing capacity withinside the combat in opposition to most cancers and require greater in-intensity research. It is thrilling that those results of plant phenolics on most cancers inhibition are much like the ones said for unique fatty acids (omega-three PUFA, conjugated linoleic acids).

While phenolic consequences are typically high quality in in vitro cells and animal models, the observations of fewer human interventions are much less clear. This is unexpected given the high quality epidemiological information and can relate to synergistic interactions among combined diets and compounds, or to the bioavailability

of person compounds. Most in vitro research with phenolic compounds used better concentrations than can be received from the diet, suggesting the function of fortified, useful meals in most cancers suppression (20).

Terpenes

Terpenoids are derived from five-carbon isoprene units and are divided into monoterpenes, sesquiterpenes, diterpenes, sesterterpenes, triterpenes, and tetraterpenes. Many herbs that are thought to have anti-cancer effects are thought to exhibit these properties due to terpenes (21). It is a monoterpene species obtained from the roots of the Madagascan plant Albizia gummifera, and A2780 has been shown to exert antitumoral effect against human ovarian cancer cells (22), while a diterpene named Cafestrol from Coffea arabica inhibits angiogenesis (24). In vitro studies showed that Xanthorrhizol, a sesquiterpenoid terpeoid complex derived from the rhizome of Curcuma xanthorrhizza, inhibits tumor formation and development (24).

Result:

Nowadays, researchers have turned to compounds with different plant-derived anticancer properties in order to develop resistance against cancer treatment agents and to reduce their harmful effects on the body. Therefore, new studies on new plants and drugs derived from plants are promising for the treatment of cancer.

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Resistance problem in *Enterobacterales*

Gülşen HAZIROLAN

Antibiotic resistance in *Enterobacterales* is occurring a problem in our country like as worldwide. *Enterobacterales* are known to survive for long periods in the hospital environment and in patients. These bacteria can spread their antibiotic resistance properties either by clonal spread or through plasmids. Among Gram negative enteric bacteria, *Klebsiella* spp., *Escherichia coli, Enterobacter* spp., *Serratia* spp., *Citrobacter freundii* and *Proteus* spp. are the most frequent bacteria in hospitalized patients, especially in intensive care unit patients. The most important antibiotic resistance problem for these bacteria is resistance to beta-lactam group antibiotics. Resistance to beta-lactams in *Enterobacterales* is mainly conferred by beta-lactamases. These beta-lactamases.can be classified as plasmid mediated rapidly spread group [TEM type beta-lactamases, Extended-spectrum β-lactamases (ESBLs)] , plazmid mediated cephalosporinases (broad-spectrum cephalosporinases not inhibited by clavulanic acid) and CTX-M type beta-lactamases (highly effective cefotaxime and ceftriaxone, sensitive to ceftazidime and cefepime, and are increasingly being detected). Chromosomal-mediated inducible β-lactamases can cause resistantance in some a *Enterobacterales.* Chromosomal-mediated inducible β-lactamases can cause resistance in *Enterobacter* spp., *C. freundii, Serratia* spp.,and *Morganella morganii*. Choosing appropriate β-lactam therapy for organisms with functional chromosomal inducible beta-lactamases genes is complicated by the risk of selecting for stably de-repressed mutants. If de-repressed mutants overexpress inducible beta-lactamases, it causes failure of cephalosporin treatments. Carbapenemase enzymes are most frequently responsible for carbapenem resistance in *Enterobacterales*. Carbapenem resistance may also be due to the coexistence of high level production of beta-lactamases such as CTX-M or AmpC with the efflux pumping mechanism, loss of porin and decrease in membrane permeability. However, their epidemiological significance is low since these resistance mechanisms are rare and not transmitted. Carbapenemases are members of the molecular class A, B, and D β-lactamases. Class A and D enzymes have a serine-based hydrolytic mechanism, while class B enzymes are metallo-β-lactamases that contain zinc in the active site. Class A carbapenemases: this class includes the chromosomally encoded SME (Serratia marcescens enzyme), NMC (non-metalloenzyme carbapenemase) and IMI (imipenem hydrolyzing beta-lactamase) enzymes. *Klebsiella pneumoniae* carbapenemase (KPC) and Guiana extended spectrum beta-lactamase (GES), which are the main enzymes transported by plasmids, are in this class. Class B carbapenemases (metallo-beta-lactamase): There are naturally produced MBLs, as well as some MBLs transferred through plasmids between species. IMP, VIM, GIM, SPM, SIM and NDM-1 (New Delhi metallo-beta-lactamase-1) enzymes are in class B. Class D carbapenemases: also known as OXA-type enzymes because they commonly hydrolyse oxacillin. Nearly 500 OXA-type enzymes found in this class. Chromosomal encoded OXA-51 and plasmid mediated OXA-23, OXA-24/OXA40, OXA-48, OXA-58, are the most frequent seen members of this class.

Aminoglycoside group antibiotics are frequently preferred in the treatment of infections caused by multidrug-resistant gram-negative bacteria due to their broad spectrum, synergistic activity with other antimicrobials and rapid bactericidal activity However, resistance to aminoglycosides is frequently observed in *Enterobacterales*, and resistance develops most frequently with aminoglycoside modifying enzymes (acetyltransferases, adenyltransferases). High levels of resistance to all aminoglycosides are also observed with target modifications (armA, rmtB, rmtC, rmtF) .Fluoroquinolone resistance in *Enterobacterales*: change in drug target (gyrA Ala67-Gln106 gyrB Asp426-Lys447 parC ,parE), decrease in drug accumulation

in the cell (decrease in membrane permeability, outer membrane porin protein changes, excretion of the antibiotic with active *active* efflux *pump*) and plasmid mediated resistance (*qnr* genes can be coexist with, SHV, CTX-M,, KPC, VEB, and OXA types beta lactamases) can be observed. In recent years, ampicillin and quinolone resistance increased in *Salmonella* spp. significantly. Genes responsible for the resistance of quinolones can be transferred by plasmids such as in many antibiotic groups. It seems that, increasing resistance rate will limit the clinical use of these antibiotics in future.

Fosfomycin is an antibiotic, that was first used in the in 1971. Due to the increasing resistance rates in *Enterobacterales* fosfomycin has been used again in the clinics. Fosfomycin resistance occurs by three mechanisms, two of which are encoded by chromosomal genes (glpT and uhpT) and one by plasmids (FomA and FomB, FosA, FosB, FosC, FosX).

Colistin is a member of polymyxins that are polycationic peptides consisting of a cyclic heptapeptide. its target is the bacterial cell membrane. Colistin is a cationic peptide and it binds to anionic lipopolysaccharides found on the outer membrane of gram-negative bacteria. Colistin displaces magnesium and calcium (ions that normally stabilize the lipopolysaccharide molecules) from the negatively charged lipopolysaccharide, leading to a loss of integrity of the membrane and an increase in the permeability of the cell envelope, leakage of cell contents, and subsequently, cell death. Colistin is recommended to be used in infections caused by multi-drug resistant *Enterobacterales*. The most common strategies for resistance to colistin are modifications of the bacterial outer membrane through alteration of the lipopolysaccharide and reduction in its negative charge. PmrA-PmrB and PhoQ-PhoP regulatory systems play a role in colistin resistance. The plasmid-mediate *mcr* colistin resistance genes, encoding the phosphoethanol amintransferase protein, were also determined. In our country, colistin resistance rates in *Enterobacterales* have been reported between 20% and 76.2%.

In order to struggle against antibiotic resistance in *Enterobacterales*, contact isolation should be applied for patients colonized or infected with multidrug-resistant *Enterobacterales* isolates that have a high risk of exogenous cross-transmission. Standard precautions such as rational antibiotic use, minimal use of invasive equipment and hand hygiene should also be considered

THE RELATIONSHIP BETWEEN COVID-19 AND ACE PROTEINS

Hande AYTUĞ[1] Funda KARABAĞ ÇOBAN[2] Alper KARAGÖZ[2]

ABSTRACT

Severe acute respiratory syndrome (SARS) Coronavirus (CoV)-2 (SARS-CoV-2) disease (COVID-19), which first appeared in December 2019, has affected the whole world in about two months. COVID-19 was announced as a pandemic by the World Health Organization on March 11, 2020. SARS-CoV-2 shares similarities with SARS-CoV, the virus responsible for the 2002–2003 SARS epidemic, and Middle Eastern respiratory syndrome coronavirus (MERS), the virus responsible for MERS. Following the SARS epidemic, researchers extensively investigated the pathophysiologic mechanisms of SARS-CoV infection, including the interaction of the virus with the heart and lungs. Based on these studies, researchers believe that the angiotensin-converting enzyme 2 (ACE2) receptor, located on alveolar epithelial cells, serves as a high affinity receptor and co-transporter for SARS-CoV-2 to enter the lungs. Medications, such as angiotensin-converting enzyme inhibitors (ACEI), block ACE2 receptors, which may predispose or protect against COVID-19 infection. This editorial summarizes the current scientific evidence surrounding this subject in order to guide clinical practice. This review summarizes current information examining the relationship between covid-19 and AGE proteins.

INTRODUCTION

In December 2019, a new infectious respiratory disease occurred in Wuhan city in Hubei province of China [1-3]. The first source of infection was linked to the Huanan seafood market, where there was intense animal contact. Subsequently, human-to-human transmission occurred and the disease called coronavirus disease 19 (COVID-19) spread rapidly in China. SARS coronavirus 2 (SARS-CoV-2), a new coronavirus closely related to SARS-CoV, has been detected in patients and has been identified as the etiological agent of new lung disease [4]. This virus originating in China has spread all over the world with its widespread contagious feature. As of September 19, 2020, the number of cases reported all over the world is 30,369,778, and the number of deaths is 948,795.

Coronaviridae family members are enveloped positive strand RNA viruses. They cause diseases in humans, other mammals and birds [5, 6]. Within the Coronaviridae, there are four subgenus called alphacoronavirus, betacoronavirus, gammacoronavirus and deltacoronavirus. Four viruses from this family (229E, OC43, NL63, and HKU1) constantly circulate in the human population, often causing mild respiratory illness similar to the common cold [7, 8]. In contrast, severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERSCoV) are transmitted from animals to humans and cause severe respiratory illness in affected individuals [9]. Contact with respiratory extracts of asymptomatic persons and patients, via droplet or direct contact is the most important route of transmission. Rapid transmission from person to person is the most important challenge in the control of the disease [10, 11].

The virus has a mean diameter of 60-140 nm, rounded and surrounded by protrusions [12]. The coronavirus genome is 40 kb, quite large for an RNA virüs [13]. The SARS-CoV-2 genome is similar to typical coronaviruses and contains 10 different ORFs (open reading frame). ORF1a / b; It encodes two large polyproteins, polyprotein / 1a and polyprotein / 1ab. Polyprotein 1a and 1ab are non-structural proteins of the virus synthesized on the basis of genomic RNA. These

two polyproteins transform into 16 nonstructural proteins (nsp1-nsp16) that make up the viral replication-transcription complex. Nsp1-16 transforms membranes originating from the endoplasmic reticulum (ER) into vesicles that will then form double-layered envelopes of the virus. Viral replication and transcription takes place thanks to the replication-transcription complex formed by nsps in these vesicles. Other ORFs code for four major structural proteins such as spike (S), envelope (E), nucleocapsid (N) and membrane (M) proteins [14]. SARS-CoV-2 genome structure is similar to beta-coronavirus type viruses. According to the genome sequence data, it is particularly similar to wild-life viruses named bat-SL-CoVZC45 and bat-SL-CoV ZXC21; It differs from SARS-CoV. It is thought that the binding of SARS-CoV-2 to the ACE2 receptor in its entry into the cell is of critical importance [13, 15].

It has been shown that SARS-CoV-2 can enter the cell by many different mechanisms, just like SARS-CoV. The first and probably the most important of these mechanisms is direct membrane fusion via ACE2 receptors. The S protein binds to ACE2 receptors and combines with the plasma membrane. The S protein belonging to SARS-CoV undergoes proteolysis after binding to the ACE receptor and this virus is an important stage in the life cycle [16]. It is not known whether the proteolysis step is required for SARS-CoV-2 infection. In another article recently published, it was observed that the SARS-CoV-2 S protein can bind to the DPP4 receptor, which MERS-CoV uses for entry into the cell, right at the MERS-CoV binding site [17]. Our knowledge about the entry mechanism of SARSCoV-2 into the cell is open to improvement. Following SARS-CoV-2 contact with cell surface elements, it is taken into the cell as viral inclusion bodies. Apart from membrane fusion, clathrin-dependent and independent endocytosis mechanisms have also been demonstrated. Positive polarity single stranded RNA genome taken into the cell is released into the cytoplasm; Transcription and translation of viral products are performed [14]. ACE2 receptors are found in many tissues such as lung, intestine, heart and kidney. ACE2 receptors are also present in endothelial cells and viral inclusions can be observed in endothelial cells after exposure to the virus. The intake and breakdown of ACE2 into the cell during virus entry causes an increase in angiotensin-2 by affecting the renin-angiotensin system (RAS). Endothelitis, apoptosis and deterioration in RAS balance resulting from the infection of endothelial cells; leads to ischemia, edema, hypercoagulability and many other conditions [18, 19]. In addition, COVID-19; It may also be associated with conditions such as stroke and hypertensive crisis that may be observed in patients [12].

RESULT

SARS-CoV-2 is still a virus that threatens the whole world and can spread very quickly. The COVID-19 it causes can lead to serious pathologies in many tissues, especially the respiratory tract, and can cause common systemic complications that can progress to multiple organ damage. There is a lack of scientific evidence and clinical data to support discontinuing ACE/ARB use in patients with COVID-19 and co-existing heart failure, hypertension, or ischemic heart disease. The well-studied reduction in mortality conferred by ACE/ARB use and the beneficial effects for patients with diabetes, chronic kidney disease, and proteinuria or albuminuria currently outweigh the theoretical risks. As the COVID-19 pandemic continues to rapidly evolve and affect more patients with cardiovascular comorbidities, further research is needed to clarify the accuracy of existing hypotheses. What will be the long-term consequences of the disease is still a matter of concern.

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Bee Pollen: Its Yeast and Mold Communities

Hidayet TUTUN[1], Hatice Ahu KAHRAMAN[2*],

Abstract

Apitherapy is a type of alternative medicine that relies on the use of honey bee products such as honey, propolis, pollen. Bee pollens, which is a natural product derived from honey bee foragers, contain a group of chemical compounds with approved action and range of activity. Bee pollen is used as a valuable source of nourishing substances due to the benefits provided by the bioactive compounds in it. Bee pollen is widely consumed as food or health supplement in developed countries at the present times. Although a lot of works have been conducted on the chemical composition, botanical evaluation, drug residue analysis and screening of biological properties of bee pollen, little is known on its safety risk associated with microbiological hazards. In addition to its chemical compounds and nutritional value, analysis of its microbiological safety will help to valorize the bee pollens. This chapter will give information about bee pollen and its mold and yeast communities

Keywords: Bee pollen, Honey bee, Microbiology, Mold, Yeast

Introduction

Bee pollen derives from flower pollens with floral nectar of certain plants and the salivary substances of bees. Honey bees utilize salivary secretion, or nectar to adhere pollen grains. Bee pollen has gained reputation as an important source of energy and beneficial substances such as terpenes, fatty acids, flavonoids, carotenoids, tocopherol, niacin, thiamine, phytosterols (Guine, 2015; da Silva et al., 2014). More and more researchers are increasing their attention towards bee pollen's beneficial properties and therapeutic use in different pathological conditions in recent years (Komosinska-Vassev et al., 2015). Bee pollen can be easily collected from returning foragers using specific systems, the use of pollen traps and is commercialized for human consumption after several stages including drying, cleaning and storage in processing pollen. Bee pollen is one of the richest and purest natural foods and is widely consumed as food or health supplement in developed countries at the present times. While a great deal of research describes its chemical composition, chemical residues, botanical origin, and other biological activities, there has been very little research on its safety risk associated with microbiological hazards (Almaraz-Abarca et al., 2004; Bernal et al., 2010; Estevinho et al., 2012; Mauriello et al., 2017). This chapter will focus on bee pollen and its mold and yeast communities.

Bee pollen

Bee pollen is a product obtained by honeybee foragers from gathering floral pollen grains and mixing it with plant nectar and honeybee saliva, and is the main ingredients forming the sustenance of a hive. Pollen is collected by honeybee foragers from various plants on one foraging trip, adhered to the outside of their hind legs and transported into the hives in the form of pellets (Margaoan et al., 2013). Bee pollen is a mixture a plant pollen with nectar, honey, enzymes, wax and honeybee secretions. It is very important in human nutrition, due to its active natural metabolites with a wide range of nutritional and therapeutic properties. The nutritional components in bee pollen include a high concentration of reducing sugars, proteins, unsaturated and saturated fatty acids, vitamins, minerals, polyphenols, the presence of Zn, Cu, Fe, and high K/Na ratio and a small percentage of other components (Almeida-Muradian et al., 2005; Campos et al., 2008; Li et al., 2018). Bee pollens contain varying amounts of proteins (more than 16 amino acids) with valine, leucine, isoleucine, phenylalanine, proline, lysine, arginine,

cysteine, tryptophan, tyrosine, methionine, histidine, glycine, alanine, threonine, serine, glutamic acid and aspartic acid depending on biogeographic origin, ecological habitat, or even the season (Gorissen et al., 2018; Taha et al., 2019).

Honey bee products including propolis, honey, bee pollen, royal jelly have been used for as alternative drugs. Currently, bee pollen has been recognized to be a valuable apitherapeutic product due to its potential bioactive and therapeutic properties (Komosinska-Vassev et al., 2015). Bee pollen has health-enhancing value due to the presence of a wide variety of secondary plant metabolites including terpenes, fatty acids, flavonoids, polyphenols, carotenoids, tocopherol, thiamine, provitamin A (β-carotene), niacin, biotin, folic acid, phytosterols, enzymes and co-enzymes (Denisow and Denisow-Pietrzyk, 2016; Jannesar et al., 2017; Nascimento and Luz, 2018). A wide diversity of primary and secondary metabolites in bee pollen exhibit a broad spectrum of biological and pharmacological properties including antioxidant (Kocot et al., 2018), anti-inflammatory (Maruyama et al., 2010), anticarcinogenic (Choudhari et al., 2013), antibacterial (Kačániová et al., 2015), antifungal (Koç et al., 2011), hepatoprotective (Yıldız et al., 2013) and immuno-enhancing (De Oliveira et al., 2013). The main challenge in applying bee pollen in modern herbal medicine is related to the wide species-specific variation in its composition. Therefore, variations may differently contribute to bee pollen properties and biological activity and hence therapeutic effects (Denisow and Denisow-Pietrzyk, 2016).

Mold and yeast communities in bee pollen

Pollen is the one of a valuable nutritional herbal product, containing carbohydrates, proteins, enzymes, fatty acids, minerals, and vitamins (Nascimento and Luz, 2018). The rich nutritional value, water content and water activity of bee pollen make it an ideal environment for the growth of various bacteria, mold and yeast. The microbial contamination of bee pollen may arise from several factors and sources such as honey bees, weather, plant materials, insects, animals, humans and agricultural devices (Hani et al, 2012). Inadequate conditions during the collection of bee pollen, its transport and storage may affect the microbial communities of the pollen. Improper storage with high humidity and prolonged storage may allow the bacteria and mold to multiply and raise the risk of contamination (Kieliszek et al 2018). In terms of the health and safety of consumers, as well as analysis of the pollen's chemical content, the analysis of its microbial composition is important. Microbiological safety is one of the most important factors determining the quality of pollen. However, there is little work on the microbiological composition of the pollen.

Water content and water activity are important for growth of bacteria, yeast and mold. The foods with high water activity (above 0.95) provide sufficient moisture to support microbial growth. Drying food below a critical water activity level provides an effective means to inhibit the growth of the organisms (Beuchat, 1983; Popa et al., 2009; Tapia et al., 2020). Fresh bee pollens have high level of water content, ranging from 20% to 30%, making them a suitable environment for mold and yeast to grow (Barajas et al., 2012). In addition to high moisture and water activity, optimum pH-value can support to the growth of them. As a result of the presence of mold and yeast, mycotoxins occur more frequently in the bee pollens. Mycotoxins are secondary metabolites of molds that can cause several harmful effects to both animals and humans, in addition to significant economic losses (Kostić et al., 2019; Keyvan et al., 2018). Human may possibly be exposed to mycotoxins directly by consumption of this contaminated foods or indirectly by consuming animal foods exposed to mycotoxins *(*Krnjaja et al., 2012*)*. Mold spores or mycotoxins could be cause acute or chronic poisoning by directly consumption of this contaminated foods.

The fresh pollen has very high moisture content (DeGrandi-Hoffman et al., 2013). The quality of bee pollen is affected from the methods applied during and after harvesting. After harvesting, the pollen has to be dried immediately and stored properly in order to prevent deterioration. Increased humidity during the storage of pollen can cause the development of mold and yeast. In generally, the pollen is dried traditionally by spontaneous way in several parts of the world. Drying the pollens outside, in the natural air flow could probably be the reason the pollen contamination with mycotoxigenic mold (Petrovic et al., 2014). Low temperature during drying of pollen can cause an increase in growth of molds which leads to the production of mycotoxins (Estevinho et al., 2012; Gonzalez et al., 2005; de Arruda et al., 2013)

Table 1. Mold and yeast genus and species discovered in bee pollens in literature

Identified genera of Mold/Yeast	**References**
Aspergillus sp.	Estevinho et al., 2012; Petrović et al., 2014; Kačániová et al., 2011; Beev et al., 2018; Nardoni et al., 2016; Altunatmaz and Aksu, 2016; Shevtsova et al., 2019
Penicillium ssp.	Petrović et al., 2014; Estevinho et al., 2012; Sinkevičienė et al., 2019; Beev et al., 2018; Nardoni et al., 2016; Kostić et al., 2017; Altunatmaz and Aksu, 2016; Shevtsova et al., 2019
Alternaria ssp.	Petrović et al., 2014; Kačániová et al., 2011; Sinkevičienė et al., 2019; Beev et al., 2018; Nardoni et al., 2016; Kostić et al., 2017; Altunatmaz and Aksu, 2016; Shevtsova et al., 2019
Cladosporium ssp.	Kačániová et al., 2011; Petrović et al., 2014; Beev et al., 2018; Nardoni et al., 2016; Altunatmaz and Aksu, 2016; Shevtsova et al., 2019
Mucor ssp.	Petrović et al., 2014; Kačániová et al., 2011; Sinkevičienė et al., 2019; Kostić et al., 2017; Altunatmaz and Aksu, 2016
Fusarium ssp.	Petrović et al., 2014; Estevinho et al., 2012; Sinkevičienė et al., 2019; Beev et al., 2018
Rhizopus ssp.	Petrović et al., 2014; Altunatmaz and Aksu, 2016; Shevtsova et al., 2019
Candida ssp.	Nogueira et al., 2012; De-Melo et al., 2015
Humicola ssp. *Mucoraceae* ssp.	Nardoni et al., 2016
Monascus ssp. *Trichothecium* ssp. *Geotrichum* ssp.	Altunatmaz and Aksu, 2016
Epiccocum ssp.	Petrović et al., 2014
Acremonium ssp.	Nardoni et al., 2016
Zygosaccharomyces ssp.	Nogueira et al., 2012; De-Melo et al., 2015
Saccharomyces cerevisiae *Rhodotorula mucilaginosa* *Cryptococcus humicola*	Nogueira et al., 2012; De-Melo et al., 2015
Hanseniaspora uvarum *Debaryomyces hansenii* *Pichia membranifaciens*	De-Melo et al., 2015

The limited number of studies conducted on yeast and mold communities in the bee pollen are given in Table 1. According to the studies, the results have shown the predominance of the mold from the genera *Aspergillus*, *Penicillium*, *Alternaria*, *Cladosporium*, *Fusarium* and *Mucor*. Toxigenic storage fungi including members of the genera *Aspergillus* and *Penicillium* may produce mycotoxins under favorable conditions and pose a serious health risk to human. The studies on the yeasts have revealed that *Candida* ssp., *Rhodotorula mucilaginosa*, *Zygosaccharomyces* ssp., *Cryptococcus humicola* and *Saccharomyces cerevisiae* are common in the bee pollens. *Saccharomyces cerevisiae* and *Zygosaccharomyces rouxii* are responsible for the deterioration of foods and *Candida norvegensis* and *Rhodotorula mucilaginosa* can cause health risks (Nogueira et al., 2012). According to the results of the studies, consumption of unprocessed and fresh bee pollen may present a risk for human health.

Conclusion

Due to the high values of humidity and good nutritional profile, the pollen could be a carrier for various microorganism especially mold which may harmful on body health. In order to benefit the all properties of pollen, its quality should be monitored properly. Also, attention should be paid to all stages from harvest to storage.

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A NOVEL DESIGN AND MANUFACTURING APPROACH OF INTEGRATION PCR MACHINE PC MODELLING

İdris KAYNAK

ABSTRACT

Infectious diseases cases, such as the most case of Covid-19 disease, have been brought recently the prospect of point-of-care diagnostic tests into the spotlight. This research study is contained the design, analysis, computer integration controlled manufacturing, and test of a prototype of a real-time convective cell frames polymerase chain reaction (RT-cfPCR) on PCR machine. Nowadays and in the future, there is an urgent need for domestic and national production for RT- (real time) PCR analysis devices for Covid-19 and cancer research. The PCR to be designed and manufactured is 96 units in total with 32 units in each section and 3 separate sections. It is the only control unit with heating unit micro-wells (0,2µl, 0,15µl) micro-liter heating units, controlled by digital infrared temperature sensor, monitored by camera and each can operate separately. The analysis results graph will be provided with software (Quant Studio 6) support for instant evaluations on the PCR machine-computer controlled touch screen-LCD integrated digital model.

1.Introduction

Micro-Electro-Mechanical Systems (MEMS) technology field developments have allowed the use of real-time polymerase chain reaction (PCR) machines in laboratory systems devices with the opportunity to analyze with microchips. Numerical measurement with digitally screen on of PCR device has pointed out progressively because of its splendid sensitivity, which is of particular utility for diagnosing and treating disease of instant response diagnosis and monitoring to therapy. For instance, in a research study, the used method was a digital measurement method was for the detection of bio-markers and applied to detect alpha-fetoprotein (AFP), which is a biomarker of hepatocellular carcinoma. The digital quantification method was implemented with modified magnetic micro particles (MMPs) and a microfluidic array chip. In the microfluidic array chip fabrication processing steps, the microfluidic array chips were used in research work consisting of a PDMS (poly-dimethyl siloxane) channel layer and a PDMS base layer [1]. Digital polymerase chain reaction (dPCR) technology has maintained a “interest subject” in the decades owing to its potentials implementation in cell biology, genetic engineering, and medical diagnostics. [2].

Today, many varieties instruments platform of PCR machine using are commercially available. There are varied brand-name types of PCR appliance analysis; real-time numerical/digital PCR (qPCR) had become some appointed technics for the limit of quantifications of gene expressions, with the 5′ nuclease assay using Applied Biosystems™ TaqMan® probes as the gold standard fluorescent reporter method of qPCR [3,4]. PCR is particularly useful find out results of gene properties, its prime advantage is that it enables the quantitative comparison of gene expression over a broad dynamic range $>10^7$-fold. In another research study, the polymerase chain reaction (qRT-PCR), which is performed with the quantitative reverse transcriptase method, is rapidly becoming a basic method in detailed researches on lung cancer. In the technique performed in this study, analysis of the transcriptional activity of tumor cells or the diagnosis and detection of tumor markers has the potential to change by improving the diagnosis and treatment of lung cancer [5]. The problem encountered in obtaining fast and delicate analysis results of PCR the self-contained timing software was seriously not enough properly or was delaying, a solution approach offer proposal in research study was presented. The designed study is designated from the perspective of photoelectric control and then the

conventional PCR machine was combined with a smartphone and PC. The lastly proposal step is an optical and electrical feedback automatic fluorescence detection system was designed to achieve quantitative real-time PCR [6].

In this research study, Covid cell was used and thirteen the reverse transcriptase reaction-RT-PCR experiments were selected according to the criteria that can be used by following generic RNA extraction protocols, common PCR platforms and usability. Using a 10-fold and 2-fold dilution series of a digitized SARS-CoV-2 cell cultured virus stock, performance was evaluated in comparison to in-house validated assays. The specificity of the study was tested using RNA extracted from cultured common human corona-viruses. All RT-PCR kits included in this study, except the Sentinel Diagnostics B E-gene RUO test (80%), presented in the study that they achieved PCR efficiencies of> 90% [7].

There is an abstract related to the requirements of the PCR tests and then going on to discuss the point-of-care POC-PCR into product detection methods, the integration of their functional components, the potential applications, and other practical issues, which are the execution of lab-on-a chip technologies. Since invention in 1986, several versions of PCR, such as the standard PCR (end-point PCR), the quantitative PCR (qPCR), and the digital PCR had been developed and afterwards operative in the field of molecular detections and diagnostics. For coronaviruses, as with other RNA viruses, a reverse transcription step takes precedence of the PCR the reverse transcriptase reaction (RT-PCR) and transcribes the viral RNA into component deoxyribonucleic acid (cDNA) [8]. Comparison of seven commercial RT-PCR diagnostic kits for COVID-19, many commercial PCR analysis kits have recently become available, but their performance has not yet been independently assessed. The primary aim of this investigation was comparison basic analytical and clinical performance of selected RT-PCR kits from seven different manufacturers company products (Altona Diagnostics, BGI, CerTest Biotec, KH Medical, PrimerDesign, R-Biopharm AG, and Seegene) The conclusions of research decision of seven commercial PCR machine is outlined, our findings of considering , we believe that all of the commercially available RT-PCR kits included in this study can be used for routine diagnostics of symptomatic COVID-19 patients [9]. The research about the multi-stage group testing improving efficiency of large-scale COVID-19 screening, they found that three-stage testing schemes with pool sizes of maximum 16 samples can test up to three and seven times as many individuals with the same number of test kits for prevalence rates of around 5% and 1%, respectively. Them propose an adaptive approach, where the optimal testing scheme is selected based on the expected prevalence rate. They are predicted of these research conclusion is, group testing schemes could lead to a major reduction in the number of testing kits required and help to improve large-scale population testing in general and in the context of the current COVID-19 pandemic [10]. Today present PCR instruments using are based on the semiconductor to reaching temperatures cycling, but continuous photographing's was not being allowed and the self-contained timing software's were seriously process delaying. Today's PCR instruments have 16, 32 and 96 units.

The designed and computer-integrated PCR machine modelling is 96 samples wells units in total with 32 units in each section and 3 separate sections. It is the only control unit with heating unit micro-wells (0,2µl, 0,15µl) micro-liter heating units, controlled by digital infrared temperature sensor, monitored by camera and each can operate separately. Instant control 10.1-inch LCD touch screen control screen will be used for the use of the PCR device from the screen and ease of monitoring the analysis software. Raspberry Pi is a single board computer, Raspberry Pi 4 8GB RAM with board CPU 1,5GHz will be mounted behind the LCD screen. Analyzes will be transferred via wireless communication Bluetooth 5.0 GHz and with the two ports USB 3.0. The PCR device will have a heated cover thermal insulation design that will provide integrated block usage variety, ease and fast-precise analysis opportunity. Software

feature to be used in PCR multiple graphs - up to four analysis graphs can be displayed simultaneously.

2. Method

2.1. PCR design properties

For precise PCR design optimization, the bio-systems blocks to be applied provide a better than gradient approach to PCR optimization. With the heating block for three separate 32x3 sections, you will have precise control over three temperature zones. This three blocks allows up to three experiments to be run simultaneously, completely independently of each other and without interference. The heating block is positioned at the design part upper section of PCR and maximum block ramp rate 4.0^0C/sec. The illustration of the 32 well block designed is shown in Figure 1 and the material is aluminum-1100 (Alloy 1100 UNS A91100, ASTM B209) for heating and thermal expansion coefficient.

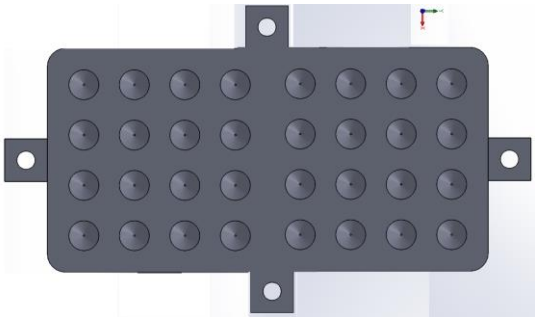

Figure 1. Sample designed view main heat-block with 32 well samples as designed (0,2µl).

It is anticipated that temperature changes will lead to corresponding changes in the device voltage indicated by V collected at a frequency of 1 kHz to record temperatures in the PCR reaction. The alternative method is that the temperature increase / decrease range of 4 degrees in the heating / cooling unit in PCR manufacturing is an important operating criterion of the system and the device. In addition, it is predicted that heat transfer between samples will be +/- 0.25^0C during analysis. The working of designed properties of PCR analysis methods for P-gene, D-gene, T-gene were assigned. These are shown as summarized in Table 1. Later on materials and methods are given as Transgenic Mouse, DNA extraction, PCR setup, Electrophoresis. The PCR design and integration properties are six distinct Peltier microchip elements that allow user to select up to six different annealing temperatures. This property allows for the possibility to evaluate multiple genes in one experiment.

Table 1. The designed properties of PCR analysis methods for P- gene, D-gene, T-gene.

P Gene	D Gene	T Gene
94°C for 3 minutes *35 cycles of:* 94°C for 30 seconds 51.7°C for 1 minute 68°C for 1 minute *Then*	94°C for 3 minutes *35 cycles of:* 94°C for 30 seconds 61.0°C for 1 68°C for 1 minute *Then*	94°C for 3 minutes *35 cycles of:* 94°C for 30 seconds 65.0°C for 1 minute 68°C for 1 minute *Then*

68°C for 2 minutes 4°C hold	68°C for 2 minutes 4°C hold	68°C for 2 minutes 4°C hold

Precise temperature control and PCR performance will be achieved by a precision thermistor and measurement circuit, sophisticated and tuned control algorithms, and the addition of a heated cover to prevent condensation. Although we significantly reduced the cost by advancing the heated lid, the heated lid was chosen because it significantly facilitates obtaining accurate PCR results, an important design goal. The design of upper PCR separation 3 x 32-well model block illustration for running up to three independent experiments simultaneously. Draft sample model design internal structure assembly and 3 x 32-well model is given in Figure 2.

(a) (b)

Figure 2. (a) PCR device internal structure assembly sketch view as solid model designed. (b) three independent 3 x 32-well model cover block draft- illustration of PCR [12 (b)].

Figure 3. The control screen of PCR software and back side seen mounted PC-main board for 10.1-inch LCD touch screen [13].

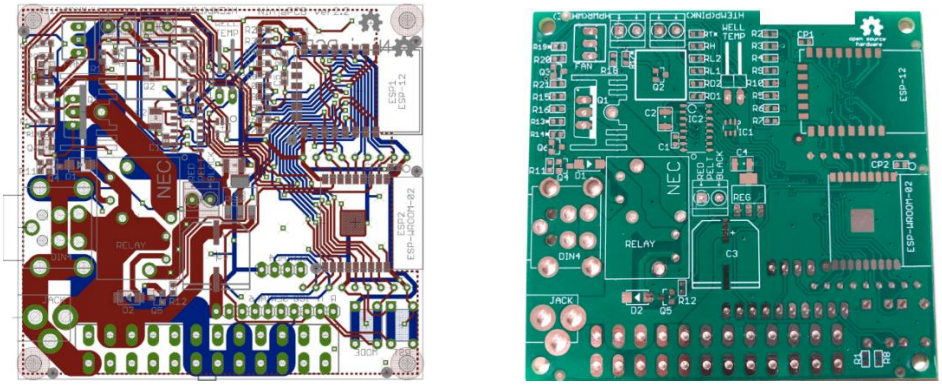

Figure 4. Printed Circuit Board (PCB) of PCR device as designed and is consist of electronic 54 tested pieces on card [14].

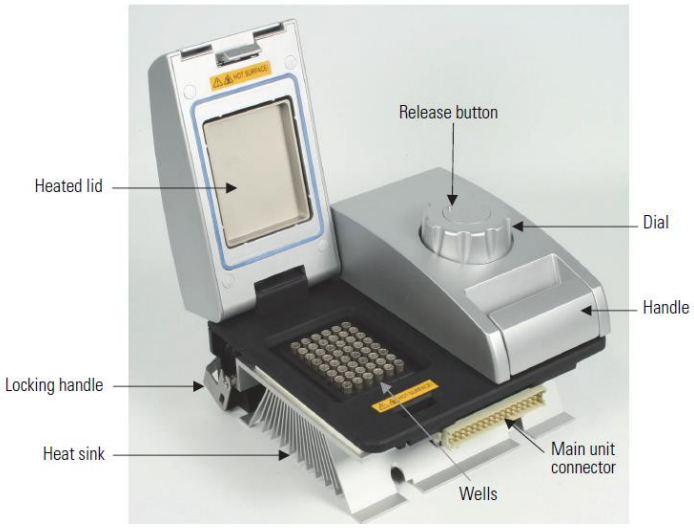

Figure 5. The design section of heated lid is modeled animation some connection parts [15].

Generally, the heating methods determine for the temperature ramping rates and are divided into contact and non-contact heating. The contact heating methods operates embedded/external heating sources, such as deposited thin films or external Peltier elements [11]; the method increases thermal mass, inevitably hindering fast thermal transitions during heat reactions. In the design of PCR will be used contact heating and cooling process.

2.2 *Temperature Control and Uniformity*

For delicate and accurate PCR analysis results, it is important that the reliable temperature of each well be consistent. The entire upper part surface area one of heat-block (aluminum-1100) has each well 0,2 µl volume and this well number there are 32 pieces. The design of heat-block began by ensuring the entire bottom surface area of the heat block interfaced with the Peltier thermoelectric unit, which simplified the thermal consistency design. The heat-block improves the thermal consistency, while the lower specific heat improves the ramp times. The small size of the thermistor responds quickly to temperature changes within the block.

2.3 *Heated Lid*

The design volume property carefully your PCR reaction mixture with the right concentrations, but it doesn't always stay that way. When the PCR machine heats up, some of what water inevitably evaporates. The small amount that initially evaporates is inconsequential, but what happens next depends on machine properties. Without a heated lid, that moisture begins to condense at the cooler, top of the tube. Eventually, this process transfers a good deal of water to a large droplet at the top of the sample tube, at which point your reagent concentrations are greatly affected, and your reaction fails. The designed of PCR machine, features an adjustable-height heated lid, which heats the tops of each tube up to 120^0C, preventing any substantial condensation. The adjustable-height lid also places a force on each PCR tube, ensuring adequate thermal contact between the PCR tube and well block.

2.4 *Experiment Profiles Management and Software Screen Entries*

A sample profile consists of one or more heat-cool cycles. A cycle has some steps and a number of repetitions. Each step has duration and temperature. If you need to cool or warm the samples very slowly, you can set the ramp time (shortest transit time) to one transition step. If the ramp time is empty, the PCR will try to change the temperature as fast as possible. If you need a

complex profile with loops with multiple patterns, use the "More Options" option to add loops. The cover temperature is 110 Celsius by default, but we can customize it to work by changing the "Heater Cover" value. Sample profile is prepared as a screenshot in Table 2 below.

Table 2. The sample of software is designated for a cycle steps experiment moderation.

Number of Cycles		Temperature/℃	Standby time
1	First Denaturation	95 ℃	2 minutes
35	Denaturation	95 ℃	30 seconds
	Annealing	55 ℃	30 seconds
	Extension	72 ℃	30 seconds
1	Last extension	72 ℃	10 minutes

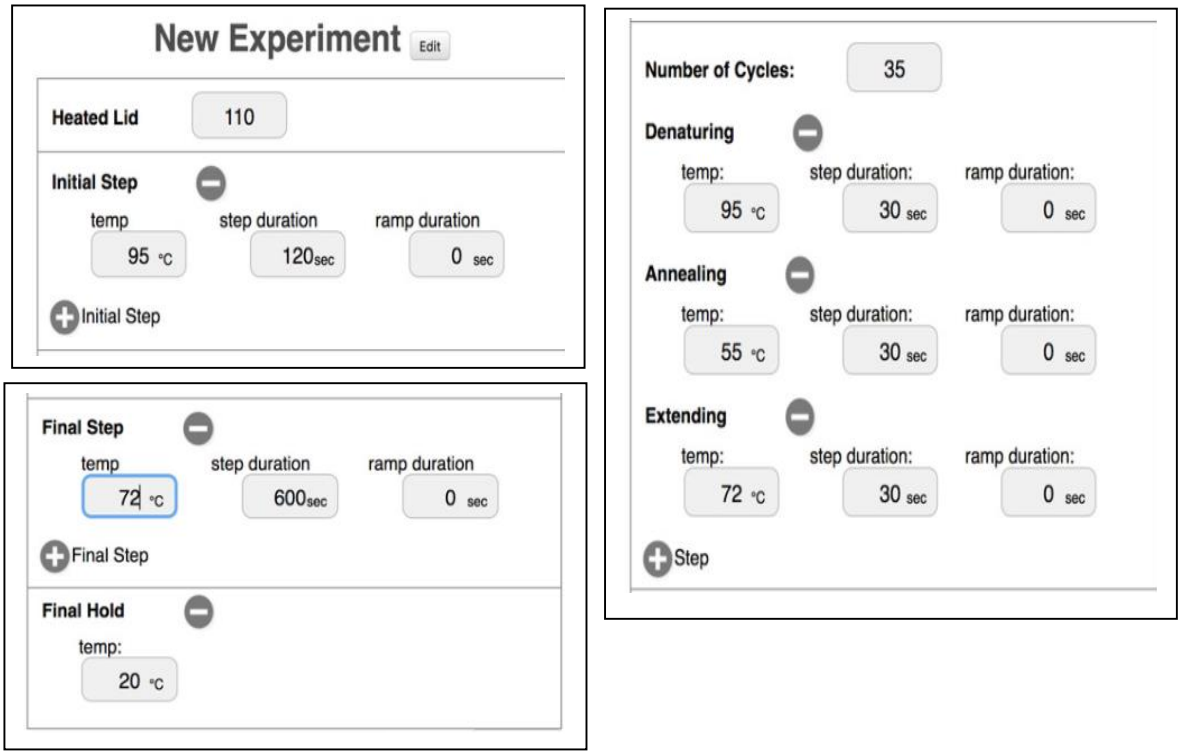

Figure 6. Software screen entries depend on Table 2. values for a cycle steps moderation [14].

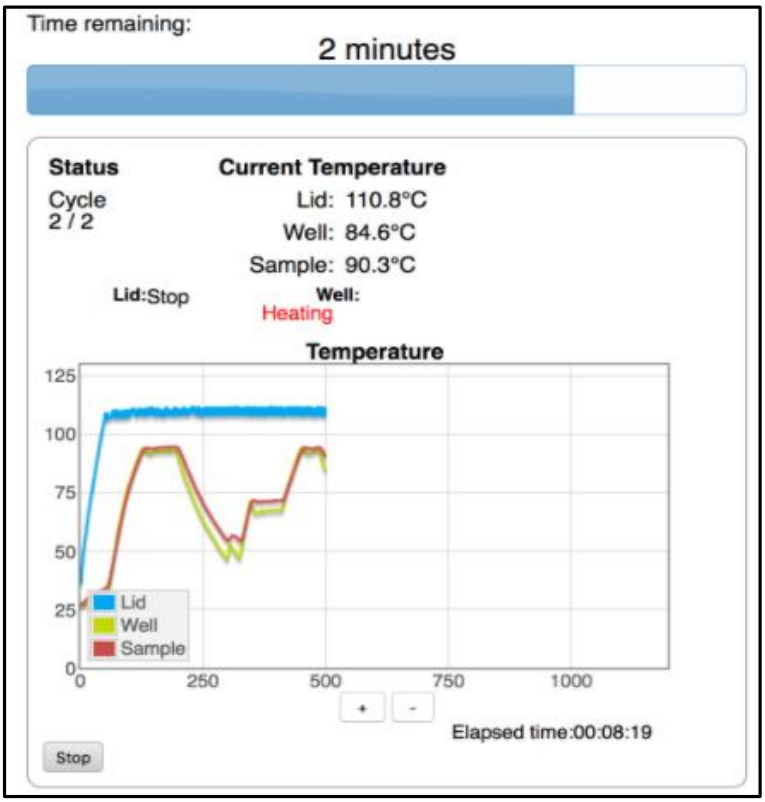

Figure 7. The sample analysis experiment steps for lid, well, sample and time remaining [14]. While the PCR is running, the console displays the well and cover temperature and the estimated temperature. It can be controlled experiment steps and graphical changes.

If it is necessary or desired to cancel the experiment, we can use the "Stop" button in the lower left corner of the screen. The total test time for the test period (Elapsed Time) is in the lower right corner. In addition, the process steps and the current action (heating-red) are displayed on the screen as a warning. It provides convenience for the time of experiment and the follow-up of the process.

3. Conclusion

Integration PCR machine A new design and production approach of PC modeling was given to Uşak University as a scientific research project-SRP. The project budget was calculated as approximately 12,600 TL (1,645 $) (1 $ = 7.66 TL), this is because some electronic components were not sold in one piece. However, considering that the international sales price of the 96-sample RT-PCR device is approximately 80,000 TL ($ 10,444), our cost advantage emerges. It is difficult to estimate the cost of the PCR device for foreign currency input to our country, considering the needs of hospitals, universities, research laboratories, ministry of health, private hospitals analysis services. The fact that there is no model that works integrated with a computer and Terabyte memory model on the PCR devices used nationally and internationally, and the ability to reach the device with conjugate / fast analysis results and the opportunity to reach the result will save time. Since the importance of time is Covid-19, the number of tests in our country has been between 7-8 million. Since the population of the country is more than 80 million, if the screening test of the whole country continues with an average of 100 thousand per day, the best probabilities and if these conditions remain constant, it will take less than 2 years. (73,000,000 / 100,000 = 730 days, 730/365 = 2 years). That is why there is a need for a

domestic and national emergency PCR device, of course, not only for Covid-19, but also for other epidemics and analyzes.

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CANCER AND ALKALOIDS

İzzet İSLAM

İntroduction

Turkey as well as in the whole world which is characterized by uncontrolled cell division and proliferation and located among the most common causes of cancer death; It is basically a genome disease that occurs as a result of changes in DNA, and then manifests itself with proteomics and metabolic changes as well as changes in the genome structure [1]. Although genetic predisposition, mutations, changes in hormones and changes in the immune system are the main factors that lead to cancer, some external factors such as smoking and alcohol use, unbalanced diet, viral and bacterial infections, chemicals, radiation and air pollution can also play an effective role in cancer development. [2].

According to global cancer statistics, cancer-related deaths worldwide are second only to heart disease with a mortality rate of 23%, followed by chronic respiratory failure, diabetes and Alzheimer, respectively [3].

All over the world, the risk of cancer and cancer-related mortality continues to increase day by day. According to the worldwide cancer statistics of 2017, approximately 1,688,780 new cancer cases are estimated, among which the cancer deaths of approximately 600,920 people are estimated [3]. These data reveal that one in four people who die every day die of cancer. Turkey Looking at the cancer statistics, the total population of 73,997,000 is being observed with approximately 90,000 deaths due to cancer; While the total number of cancer-related deaths in males is 58,400, this number is 32,500 in females. As can be understood from the figures, particularly in recent years, cancer has become critical and only a major health problem for the entire world, not for Turkey. [4].

Although the incidence and mortality rate of cancer varies depending on various epidemiological factors such as gender, age, diet, alcohol consumption, smoking, environmental and genetic factors; Among the cancer types, lung cancer has the highest incidence and mortality rate worldwide, followed by colorectal cancer, breast cancer and prostate cancer, respectively [4].

Although not statistically situation in Turkey is very different from the world; There are some differences between the sexes. Cancer with the highest mortality rate among all cancer types; lung, tracheal and bronchial cancer (32.2%), while breast cancer (15.7%) is in the first place in women [4].

It is important to diagnose and diagnose cancer in the early stages in controlling cancer by reducing the mortality rates due to cancer and increasing the survival time of patients. The methods used in cancer treatment are generally; chemotherapy, radiotherapy, immunotherapy, hormone therapy, targeted therapy and finally, surgical intervention [5].

Although a combination of one or more of these methods is preferred in the treatment, some undesirable side effects are observed in patients from time to time. There is a need for new treatment approaches that are more selective and effective that target only the relevant cancer cells, with minimizing toxic side effects. For this reason, in recent years, under the name of alternative medicine, there has been a tendency towards herbal treatments with natural and / or secondary metabolites containing anti-cancer properties.

By determining effective cancer treatment strategies for target cancer cells, in order to diagnose cancer in early stages, the changes in the protein mechanism as a result of the metastasis of the relevant cancer should be determined precisely and protein biomarkers should be determined [6].

Factors Affecting Cancer Formation

There are many factors such as radiation, heat, sunlight, industrial substances, chronic irritation, diet, smoking, alcohol, virus, stress, a sedentary lifestyle, being over the age of 55, high blood pressure, and immunodeficiency. It is known that in addition to environmental factors, genetic factors are also important in cancer formation.

Cancer incidence rates and profiles differ according to developed and underdeveloped countries. In developed countries, lung and prostate cancers are seen more in men, and breast cancer and colorectal cancers in women, while in underdeveloped countries lung, stomach and liver cancer in men, breast and cervical cancer in women are more common. Turkey "is also in male lung, bladder and stomach cancers are common, while in women breast cancer and colorectal cancer has been reported more frequently observed [7].

Cancer and the Cell Cycle

Many mechanisms play a role when a normal cell turns into a cancer cell. However, not all of these mechanisms have been fully discovered. Before trying to understand how these mechanisms come about, we need to know what the life cycle of a normal cell is like. The life cycle of the cell (cell cycle) is the program of cell growth and proliferation. It is divided into two as a period of rest and division. Vital activities continue during the rest period. It is a longer period than the cleavage period. This period is also called the GO phase. The cleavage period consists of 4 basic phases.

These phases consist of the G1, S, G2 phases where preparation for division is made and the M phase where mitosis takes place. A biochemical cycle is started with external stimuli. At the beginning of the cycle, it reproduces two identical cells that will double the cell content. During the cell cycle, some oncogenes and cell cycle specific proteins are activated and inactivated simultaneously [8].

In the G1 phase, the RNA and proteins required for DNA replication are first produced, while in the S phase, the regions where DNA replication will begin are found and marked. The DNA is paired so that it is made diploid. In the G2 phase, final preparations are made for mitosis to occur. The M phase is the mitosis division phase. In other words, it is the phase in which a cell becomes two identical cells. There are many complex parameters and interactions that regulate

and control the cell cycle. As a result of errors in the cell cycle control mechanism, cell division control is lost, resulting in cancer development [9].

Alkaloids

Alkaloid are called alkaline substances that carry nitrogen in the ring and generally show strong physiological and pharmacodynamic activity. Alkaloids play a role in the defense of plants against herbivores and pathogens. There are about 12,000 alkaloids known for their biological activity. Alkaloids are used as pharmacological, stimulant, narcotic and poison [10,11]. Alkaloids are generally found in flowering plants, less frequent flowers and some animals [loquat beaver and salamander] [12]. Alkaloids are usually collected in certain organs of the plant and not all organs may contain alkaloids.

For example, a poppy plant contains alkaloids in its capsule, while its seeds do not contain alkaloids. In addition, plants have more than a single alkaloid type, but more alkaloid groups with the same basic structure. One of them is more or more active than the others [13]. Alkaloids, which have complex molecular structures, usually contain at least one nitrogen atom in the amine structure. Hydrocarbon groups consisting of carbon and hydrogen are attached to the nitrogen atom, and the amine structure is often contained in a ring structure on nitrogen or hydrocarbon groups. Alkaloids are more common in solanaceae (Soeleneceae), weasels (Papaveraceae) and madder (Leguminoceae) families. Plants containing alkaloids contain more than 0.01% alkaloids [14].

Biochemical studies of alkaloids in plants began in 1806 with the isolation of morphine. Due to the stereo-chemical complexity of the morphine molecule, its structure could not be explained until 1952. For more than half a century, alkaloid biosynthesis in plants has been tried to be understood through chemical, biochemical and molecular research [15].

Alkaloids often cause poisoning in animals and humans. An alkaloid contained in Cotalaria and Heliotropium species causes liver cirrhosis when taken continuously. The alkaloid produced by a type of fungus known as rye burs found in cereal seeds causes ergotism. Physiological effects of alkaloids are important in medicine. For example, morphine obtained from poppy is used as a pain reliever in medicine, and noscapine is used as an antitussive [12].

The intake amounts of alkaloids are also important. Alkaloids taken in small amounts can be beneficial, while alkaloids taken in large amounts can be lethal [13]. The poisonous cone alkaloid obtained from hemlock causes death by paralyzing the respiratory tract when used excessively. Misuse of methadone used in medicine also causes addiction. Therefore, the use of the drug beyond the doctor's control poses serious dangers to humans [16].

Result

In addition to the development of resistance against cancer therapeutic agents from past to present, researchers have turned to compounds that are thought to have different plant-derived anticancer properties in order to reduce their harmful effects on the body. In the context of this information and researches, new studies on new plants and medicines derived from plants provide a different perspective in cancer treatment, and give hope to new methods and advances in treatment.

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SARS-CoV-2 Vaccine Development Studies

Irmak BARAN

It was reported that on December 31, 2019, many pneumonia cases of unknown etiology were occurring in Wuhan, China (1). Following this, there was a rapid increase in the number of cases, and in mid-March, it was announced that there were more than 80 thousand infected people and more than 3 thousand deaths in China. It has been found that the disease is caused by a new coronavirus, which is causing severe acute respiratory syndrome (SARS). This new virus was defined as SARS-CoV-2 and the disease it caused as COVID-19 (1,2).

The World Health Organization declared the novel coronavirus disease (COVID-19) that emerged in 2019 as an International Public Health Emergency on January 30, 2020, and as a pandemic on March 11, 2020. The number of COVID-19 cases has increased surprisingly around the world, contrary to what was initially expected. As of September 21, 2020, the total number of confirmed cases increased to 31,280,603 and the number of deaths to 965,668 (1,3).

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is the causative agent of the ongoing pandemic, belongs to the Betacoronavirus (β-CoV) genus of the Coronaviridae family (1, 4). Coronaviruses are essentially common human pathogens; two types of Alphacoronavirus (229E and NL63) and two types of Betacoronavirus (OC43 and HKU1) are circulating in humans and cause seasonal colds (1).

The SARS-CoV-2 name is given because the genomic sequences of this current virus resemble the SARS (severe acute respiratory syndrome) virus previously described in 2003 (1). SARS-CoV-2, together with severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome associated coronavirus (MERS-CoV), constitute the three most life-threatening species among all human coronaviruses (4).

SARS-CoV-2 has a linear single-stranded RNA genome with positive-polarity. This genome encodes 4 structural proteins [spike (S), envelope (E), membrane (M) and nucleocapsid (N)], 16 nonstructural proteins (nsp1-nsp16), and several accessory proteins.

The virus uses surface spike protein to bind to ACE-2 (angiotensin converting enzyme-2) receptors on the surface of human cells (1,5). Proteolytic cleavage of the S protein and fusion of viral and endosomal membranes trigger the release of viral RNA into the cytosol (1).

Since this is a very recently encountered virus, there is no specific antiviral drug or vaccine against SARS-CoV-2. Therefore, the urgency in the development of vaccines is vital to stop the pandemic and prevent new viral outbreaks (1,4).

SARS-Cov-2 Vaccine Development Strategies

During the 2009 H1N1 influenza virus epidemic, vaccine manufacturers rapidly transformed their production lines from producing trivalent seasonal influenza virus vaccines to producing monovalent pandemic vaccines. For this, basically only the types of production were changed and established and approved production processes, established release criteria and existing and approved quality control processes were used. However, it took 6 months for the vaccine to be ready to be distributed and used. This time, a new challenge awaits humans in the form of a nascent virus, and our response will be more complex because there are currently no vaccines or manufacturing processes for coronaviruses (1).

After the genetic sequence of SARS-CoV-2, the coronavirus that causes COVID-19, was published on 11 January 2020, intensive global R&D activities were initiated to develop a vaccine against the disease (6,7). The first vaccine safety trials on human subjects began in March, but the path ahead remains uncertain. Some of the attempts being made so far will fail and some others will end without a clear result. However, it is possible that very few of them will be successful in stimulating the immune system to produce effective antibodies against the virus (7,8).

Vaccines typically require years of research and testing before they enter clinical use, but scientists are currently competing to produce a safe and effective coronavirus vaccine (7,8). As of September 2020, 231 vaccine development studies have been carried out against SARS-CoV-2 by research teams in various companies and universities around the world (2, 6). Most of them are still in the preclinical, animal experiment stage. Of these, 40 are in the clinical trial phase in human subjects, and nine of them have reached the Phase III phase (2,7). There is no fully approved vaccine so far. There are vaccines approved for limited use (7,8). Researchers are experimenting with many different technologies, some of which have not previously been used in a licensed vaccine (5).

New Vaccine Development Steps

According to the CDC (Centers for Disease Control and Prevention), the general steps in the development of a new vaccine are defined as follows (9)

1. Exploratory stage
2. Pre-clinical stage
3. Clinical development
4. Regulatory review and approval
5. Manufacturing
6. Quality control

In the preclinical stage, scientists test a new vaccine on cells and then give it to animals such as mice or monkeys to see if it induces an immune response. There are currently 92 validated preclinical vaccines that are in active development (7,8).

Clinical trials are a three-step process.

1. During **Phase I**, a trial vaccine is given to approximately a few dozen small groups of people (between 20-100 healthy volunteers) to confirm that it stimulates the immune system as well as conducting safety and pre-dosing research. Phase I is essentially a safety study of a vaccine. This stage can normally take months.
2. In **Phase II**, the clinical trial is expanded and individuals with characteristics like the population targeted by the new vaccine (such as age and physical health) are

vaccinated. Phase II studies are carried out after the success of phase I is shown. Typically, the immunogenicity, dose levels and possible adverse effects of the candidate vaccine are determined by the administration of the vaccine on hundreds of people. Phase II is known as the extended study. The vaccine is given to special groups such as children and the elderly to look at the effects on different groups. While this stage may take months, it can take up to two years. In phase I-II studies, pre-safety and immune response potentials are tested.

3. In **Phase III**, the vaccine is delivered to thousands of people and tested for efficacy and safety. In addition to increasing the number of subjects in phase III studies, a control group is also included. It is observed how many of those who received the actual vaccine were infected compared to volunteers who received a placebo. These trials can determine whether the vaccine offers protection against coronavirus. In June, F.D.A. said that for a coronavirus vaccine to be considered effective, it must protect at least 50% of the vaccinated people. While the effectiveness of the vaccine is tested, side effects that may occur at the optimal dose are also observed. Phase III is known as the efficacy study. While this stage may take 1-5 years, or in some cases, this period may take longer (2, 7-10).

Combined (Abbreviated) Stages: One way to speed up vaccine development is to combine the phases. Normally, the time required to produce a vaccine is about 20 years. In order to shorten this time, controlled challenges can be carried out, which provide an accelerated path by by-passing typical Phase III trials, following the successful conclusion of preliminary safety and efficacy studies of a vaccine candidate in laboratory animals and healthy humans. Similar acceleration studies have been applied in diseases that are less deadly than COVID19 infection, such as influenza, typhoid, cholera and malaria (2). For some coronavirus vaccines, Phase I-II and Phase II-III trials are currently being conducted at the same time, for example for the first time vaccine is being tested on hundreds of people without being tested on a limited number of individuals.

Early or Limited Approval Phase: China and Russia have approved vaccines for use in certain populations without waiting for the results of Phase III trials. However, experts say there can be serious risks in rushing the process.

Approval Phase: The regulatory authorities of each country review the vaccine trial results and decide whether the vaccine will be approved. During a pandemic, the vaccine can obtain emergency use before it receives official approval. After a vaccine is licensed, researchers continue to monitor the recipients of the vaccine to make sure it is safe and effective (7,8).

SARS-CoV-2 Antigens that can be Selected for Vaccine Development Studies:

1. Whole cell antigens (WCA):

Whole cell antigens (WCA) include all proteins, lipids, polysaccharides, nucleic acids and some other elements of the virus. WCA is used to develop dead whole cells and attenuated live vaccines. Its disadvantage is that it is difficult to evaluate quality control and consistency as it has a complex structure. Until now, SARS-CoV-2 virus strains have been successfully isolated in many institutes and the development of a dead or live attenuated vaccine based on the use of whole cell antigens has been started. However, another issue to be considered in the

development of such vaccines is the need to use strains with extremely low or no pathogenicity (11).

2. Spike protein (S protein):

The S protein currently appears to be the most promising antigenic structure for SARS-CoV-2 vaccine development. Because it can be easily recognized by the host's immune system since it is the structure on the surface of the virus. It plays a role in the entry of the virus into the target cell by interacting with ACE-2 receptors on the host cell surface. Vaccine development studies have been carried out against its counterparts in SARS-CoV and MERS-CoV (11, 6). The S protein monomer found in SARS-CoV-2 contains 1273 amino acids and has a molecular weight of 140 kDa. The S protein has two subunits called S1 and S2. The S1 subunit also consists of two parts, the N-terminal domain (NTD) and the C-terminal domain (CTD). The receptor binding domain (RBD) is located on the CTD. The S2 subunit contains basic structures such as the internal membrane fusion peptide (FP) responsible for membrane fusion. Possible regions of the S protein that can be used to develop vaccine are full-length S protein, RBD domain, S1 subunit, NTD and FP.

a. Full-length S protein: Since it has more epitopes and may show higher immunogenic properties.

b. RBD: Since the RBD part of the S protein directly interacts with the ACE-2 receptor on the host cells, specific antibodies induced by RBD immunization can prevent this recognition, thereby effectively prevent the virus invasion. The RBD domain has also been used in vaccine studies developed against SARS-CoV and MERS-CoV. It has been reported that the RBD domain is relatively well conserved compared to the S1 subunit and contains multiple conformational neutralizing epitopes. This makes RBD a more suitable candidate for vaccine development.

c. NTD: N-terminal domains of various coronaviruses have been reported to show binding activity to carbohydrate receptors. While the function of the S1-NTD of SARS-CoV-2 has not yet been fully discovered, it is thought that it may play a role in binding certain receptors and therefore may be a candidate antigen for vaccine development.

d. S1 subunit: It contains both RBD and NTD. It is involved in binding the S protein to host cell receptors. It is widely used in vaccine development studies.

e. FP: The FP domain of the S2 subunit is involved in the membrane fusion of the virus, which is an important step in viral pathogenicity. Therefore, it is a candidate antigen for vaccine development (11).

3. Nucleocapsid protein (N protein): The N protein is the most abundant protein in the coronavirus and normally contains highly conserved regions and has a molecular weight of about 50 kDa. The N protein has many functions, including the formation of nucleocapsids, virus budding, RNA replication and mRNA transcription. This protein has been reported to be highly antigenic. It can be used as a marker in diagnostic tests due to its high immunogenicity (11).

4. Membrane protein (M protein): M protein is a transmembrane glycoprotein with a molecular weight of about 25 kDa and plays a role in the assembly of virus fragments and it has been determined that this protein is the most abundant protein on the surface of SARS-CoV which is a virus from the same family. Immunogenic and structural analyzes have shown that the transmembrane region of the M protein contains a T cell epitope cluster capable of inducing a potent cellular immune response. The M protein is also highly conserved during evolution

between different species, so it could be a candidate antigen for developing the SARS-CoV-2 vaccine (11).

5. Envelope protein (E protein): Studies have shown that the envelope protein is not suitable for use as an immunogen (11).

Structural Types of SARS-CoV-2 Vaccines Under Development

1. Inactivated or live attenuated whole cell vaccines

Inactivated or live attenuated whole-cell vaccines present multiple antigenic components to the host, thereby potentially inducing various immunogenic effectors against the pathogen. They are traditional vaccines whose preparation technologies are well-studied and have the potential to be the first SARS-CoV-2 vaccine to enter clinical practice. Viral deoptimization is used to synthesize live attenuated vaccines. This technology starts with viral genome sequencing and allows the rapid generation of multiple vaccine candidates against the virus (8,11). In live attenuated vaccines, a virus is attenuated by regular passaging into animal or human cells until it acquires mutations that may cause less disease. In inactivated virus vaccines, the infectiousness of the virus is eliminated by using heat or chemicals such as formaldehyde (5). Advantages of this type of vaccine are it uses simple procedures previously used for various licensed human vaccines so existing infrastructure can be used. Immunogenicity can be increased by using adjuvants. Disadvantages: Generation of infectious clones for attenuated coronavirus vaccine is time consuming due to genome size. Security tests must be comprehensive. For inactivated vaccines, it is necessary to start with large amounts of infective virus initially. Antigen and/or epitope structure must be confirmed before use (1,5). Currently, many research institutions have started research on these vaccines (11).

2. Protein vaccines

Many researchers also aimed to develop a vaccine by introducing coronavirus proteins directly into the body. Protein fragments that mimic the outer surface of the coronavirus or proteins in the form of a hollow shell can be used for this (5,8). Advantages of this type of vaccine: The infectious virus does not need to be manipulated; adjuvants can be used to increase immunogenicity (1). Licensed vaccines based on recombinant proteins are available for other diseases and their production capacity can be utilized (6). Disadvantages: Global production capacity may be limited. Antigen and/or epitope structure must be verified before use. Their efficiency should be high enough (1).

a. Protein subunit vaccines

Subunit vaccines contain one or more antigens with strong immunogenicity that can effectively stimulate the host immune system. Generally, this type of vaccine is safer and easier to manufacture, but generally requires the addition of adjuvants to achieve a strong protective immune response. Until now, several institutes have initiated programs on the SARS-CoV-2 subunit vaccine, and almost all use the S protein or its RBD domain as the antigen (5,8,11).

b. Synthetic peptide or epitope vaccines

These vaccines contain only certain fragments of intact antigens and are usually prepared by chemical synthesis techniques. They are easier to prepare and quality

control. However, the low molecular weight and structural complexities of these vaccines often result in low immunogenicity, so it is necessary to add structural modifications and adjuvants to their formulations (8,11).

c. Virus-like particles

Hollow virus shells that mimic the coronavirus structure are used. These are not contagious because they lack genetic material. It is assumed that they will induce a strong immune response. However, their production is difficult (5,8).

3.Nucleic acid vaccines

It has been reported that at least 20 teams are in vaccine studies with a nucleic acid structure (in DNA or RNA form) for coronavirus proteins that causes an immune response. The aim here is to introduce nucleic acids that replicate virus proteins into human cells. Most of these vaccines encode the spike protein of the virus. RNA and DNA-based vaccines are safe and easy to manufacture only genetic material, not viruses, is sufficient to produce them. However, this technology has not yet been fully proven: this technology is not used in any licensed vaccine yet (5). Platforms based on DNA or mRNA have the potential to alter SARS-CoV-2 antigen functions to generate a stronger immune response. They can be quickly evaluated, improved for long-term stability, and prepared for large-scale production capacity (2,6,8,11).

a. mRNA vaccines

In the last two decades, with the development and maturation of mRNA synthesis, modification and delivery technologies, research on mRNA vaccines has again gained interest. mRNA vaccines represent a promising alternative to traditional vaccine approaches due to their high potency, short production cycles, low cost production and safe application. Development processes of mRNA vaccine include selection of antigens, optimization of sequences, screening of modified nucleotides, optimization of delivery systems, evaluation of immune response, and safety testing (8,11). RNAs are often sent in a lipid jacket so that they can enter cells (5). Since no mRNA vaccine has yet entered the market, it is thought that establishing quality standards and safety evaluation may take a long time (2,8,11). Advantages of RNA vaccine: Infectious virus does not need to be manipulated; vaccines are typically immunogenic, rapid production is possible. Disadvantages: Safety issues related to reactogenicity have been reported (1).

b. DNA vaccine

DNA vaccines typically consist of DNA plasmid molecules encoding one or more antigens (8,11). Through a process called electroporation, pores are created in membranes to facilitate uptake of DNA into a cell (5). They are superior to mRNA vaccines due to the stability and transmission efficiency in their formulations; however, they may cause vector integration and mutation risk in the host genome as they must enter the nucleus (11). Advantages: The infectious virus does not need to be manipulated, can be produced in large amounts easily, production costs are low, have high heat stability, fast production is possible. Disadvantages: The vaccine needs mediator specific molecules to produce good immunogenicity (1).

4. Live vector vaccines

Live vector vaccines are live viruses (the vectors) that express a heterologous antigen. These have the combination of the strong immunogenicity of live attenuated vaccines and the safety of subunit vaccines and are widely used to induce cellular immunity in vivo (8, 11). Often a virus such as measles virus or adenovirus is used. With genetic engineering, it is ensured that these viruses can produce coronavirus proteins. Since these viruses are weakened, they do not cause disease themselves. There are two types of viruses that can be used for this task: those that can still replicate inside cells (the replicating viral vector; such as attenuated measles virus) and those that cannot replicate because their key genes are inactivated (non-replicating viral vector; such as adenovirus) (5). Advantages: No infectious virus needs to be manipulated, previously studied for many new viruses, including MERS-CoV. Vaccines based on viral vectors offer high levels of protein expression and long-term stability, and strongly induce the immune response (1, 6). Disadvantages: Immunity to the vector can adversely affect vaccine efficacy (depending on the vector selected) (1,8).

Adjuvant

Although adjuvants do not have their own immunogenic properties, they are agents that increase the immunogenic properties of the molecules they are with. In addition to live attenuated vaccines and vector vaccines, adjuvants can be used to increase the immune response to other types of vaccines (11).

For some vaccine development technologies, adjuvants may increase immunogenicity and administration at lower doses can be provided. This can enable more people to be vaccinated without sacrificing protection. To date, at least 10 developers have committed to develope adjuvanted vaccines against COVID-19 and to offer licensed adjuvants (AS03, MF59 and CpG 1018 respectively) that can be used by other vaccine developers, including GlaxoSmithKline, Seqirus and Dynavax (6).

Nonspecific Vaccines

Some vaccines have heterologous effects, also called nonspecific effects. So, this means they can have benefits beyond the diseases they prevent. For example, the BCG vaccine has been the subject of research in this regard, based on the claim that COVID-19 mortality is lower in countries where BCG vaccination is routinely. Various countries are planning randomized studies on this issue on active volunteer healthcare workers. Similar studies have been planned with the MMR (measles-mumps-rubella) vaccine (2).

Candidates for SARS-CoV-2 Vaccine Under Development

a) Some SARS-CoV-2 vaccine candidates currently in preclinical study (7,8,12):

1. French pharmaceutical company Sanofi is developing an mRNA vaccine in partnership with Translate Bio. They announced that they are planning phase I trials in the fall.

2. Other nucleic acid vaccines in preclinical development: Applied DNA Sciences, EvviVax and Takis Biotech; Chula Vaccine Research Center; DIOSynVax; Elixirgen Therapeutics; Entos Pharmaceuticals; ETheRNA; Infectious Disease Research Institute and Amyris; Mediphage Bioceuticals; the OPENCORONA Consortia; Scancell; the Spanish National Center for Biotechnology and the Spanish National Research Council.

3. Merck is partnering with the IAVI company for a second viral vector vaccine that can potentially be taken orally. Vesicular stomatitis viruses that Merck has successfully used to produce the first approved vaccine for Ebola are used. Phase I studies are planned for late 2020.

4. Swiss company Novartis will produce a gene therapy therapy-based vaccine developed by the Gene Therapy Program at Massachusetts Eye and Ear Hospital, Massachusetts General Hospital and University of Pennsylvania. It is aimed to carry coronavirus gene fragments into cells using a virus called Adeno-associated virus. Phase I trials are scheduled to begin in late 2020.

5. The vaccine Vaxart is developing is an oral tablet-style vaccine containing an adenovirus containing coronavirus genes. In September, they reported that they were able to produce neutralizing antibodies in mice and at the end of the month they would begin recruiting volunteers for a Phase 1 trial.

6. Other live vector vaccines in preclinical development: Altimmune; the German Center for Infection Research; Icahn School of Medicine at Mount Sinai; Intravacc; the Israel Institute for Biological Research; KU Leuven; Meissa Vaccines; NantKwest; the Spanish National Center for Biotechnology and the Spanish National Research Council; Thomas Jefferson University and Bharat Biotechnology; Tonix Pharmaceuticals; University of Pittsburgh; Vivaldi Biosciences; Washington University.

7. After the SARS epidemic in 2002, researchers at the Baylor School of Medicine started developing a vaccine that could prevent a new epidemic. However, despite promising early results, vaccination studies were halted when support for the research discontinued. Because the coronaviruses that cause SARS and Covid-19 are remarkably similar, the researchers revived the project in a collaborative effort with Texas Children's Hospital. Researchers found that the Covid-19 vaccine produces antibodies in mice. In August, the Indian company Biological E licensed this vaccine, saying it could potentially produce one billion doses a year.

8. A vaccine in development by the University of Pittsburgh, called PittCoVacc, is a skin patch tipped with 400 tiny needles made of sugar. When the needles are placed on the skin, they dissolve, allowing the virus proteins to spread throughout the body. Clinical trials are planned to begin in late 2020.

9. Other protein-based vaccines in preclinical development: Adaptive Phage Therapeutics; AdaptVac and Bavarian Nordic; Applied Biotechnology Institute; Artes Biotech; Axon Neuroscience; BiOMVis and University of Trento; City College of New York and TechnoVax; EpiVax; GeoVax; Heat Biologics; IBio and CC-Pharming; Icosavax and University of Washington; ImmunoPrecise Antibodies; IMV; Instituto Butantan; Intravacc; IrsiCaixa; Izmir Biomedicine and Genome Center; Navarrabiomed; NidoVax; OncoGen; Oragenics; OSE Immunotherapeutics; Osivax; PDS Biotechnology; Pontifical Catholic University of Chile; Saiba; SK Bioscience; Symvivo; University of Alberta; University of Georgia and EpiVax; University of Saskatchewan and VIDO-InterVac; University of Virginia; UNSAM-CONICET; Vaxform; Vaxil-Bio; VBI Vaccines; Verndari; VIDO-InterVac; Voltron Therapeutics; Walter Reed Army Institute of Research; Wyss Institute and Harvard University; Yisheng Biopharma.

10. Other inactivated or live attenuated virus vaccines under preclinical development: the Chumakov Center at the Russian Academy of Sciences; Codagenix; Valneva; Vivaldi Biosciences; Washington University; Western University.

11. Other recycled vaccines are in clinical trials conducted by: Bandim Health Project; Hôpitaux de Paris; Louisiana State University Health Sciences Center New Orleans; BADAS Study (Texas A&M University, Baylor School of Medicine, M.D. Anderson Cancer Center and Cedars-Sinai Medical Center); Indian National Tuberculosis Research Institute; BCG-CORONA (UMC Utrecht and Radboud University); University of Campinas; University Health Network, Serum Institute of India, Max Planck Institute for Infection Biology and Verity Drugs; Oklahoma Medical Research Foundation and the University of Oklahoma; Vakzine Projekt Management.

b) SARS-CoV-2 vaccine candidates currently in clinical trial (7,8,12):

Phase I:

1. Imperial College London researchers have developed a "self-replicating" RNA vaccine that boosts the production of a viral protein to stimulate the immune system. They started Phase I-II trials on June 15 and partnered with Morningside Ventures to form a new company called VacEquity Global Health to manufacture and distribute the vaccine. Researchers expect to find out if the vaccine is effective by the end of the year. (Phase I-II combined phase).

2. On June 30, the Japanese biotech company AnGes announced that it has begun Phase I trials on a DNA-based vaccine developed jointly with Osaka University and Takara Bio. They started recruiting subjects for clinical trials at the end of August. (Phase I-II combined phase).

3. California-based company Arcturus Therapeutics and Duke-NUS School of Medicine in Singapore have developed an mRNA vaccine. It has a "self-replicating" design that leads to the production of large amounts of viral proteins. Animal trials have shown that it is protective against infection. Phase I-II trials started at the Singapore General Hospital in August. (Phase I-II combined phase).

4. The American company Inovio has developed a DNA-based vaccine that is applied electrically to the skin using a manual device. They released preliminary data of the phase I trial in June. They said that there were no serious side effects and that 34 of 36 volunteers developed an immune response.

5. The Korean company Genexine developed a DNA-based vaccine and started safety testing in June.

6. In June, Chinese researchers at the Academy of Military Medical Sciences, Suzhou Abogen Biosciences, and Walvax Biotechnology announced that they would begin their country's first safety trials on an mRNA-based vaccine called ARCoV. They reported that protective effects were detected in preliminary studies on monkeys.

7. Italian biotech company ReiThera has developed a Covid-19 vaccine called GRAd-COV2 using an adenovirus that infects gorillas. In collaboration with the Lazzaro Spallanzani National Institute for Infectious Diseases in Rome, they launched a Phase I trial at the end of July.

8. The American company Merck acquired the Austrian company Themis Bioscience in June and started working on a vaccine developed mainly at the Institut Pasteur. A weakened measles

virus carrying the coronavirus spike protein gene is used in the vaccine. Researchers started a Phase I trial in August.

9.In 2019, researchers at the University of Hong Kong and Xiamen University produced a nasal spray-style vaccine based on a genetically attenuated form of the influenza virus. Earlier this year, they designed the same vaccine to produce part of the coronavirus spike protein. On September 9, they received approval to start clinical trials in partnership with Beijing Wantai Biological Pharmacy.

10. **NVX-CoV2373 vaccine:** Maryland-based Novavax prepares vaccines by attaching proteins to microscopic particles. The company launched trials for a Covid-19 vaccine in May. On August 4, Novavax announced that two preliminary studies in monkeys and humans showed promising results. They launched Phase II trials in South Africa on August 7. The blind, placebo-controlled study of 2,900 people is expected to measure not only the safety of the vaccine, but also its efficacy. In September, Novavax made an agreement with the Serum Institute of India, a major vaccine manufacturer, aiming to produce 2 billion doses per year. (Phase I-II combined phase).

11. On August 18, the head of epidemiology at the Cuban public health ministry announced that the Finlay Vaccine Institute in Havana will launch a clinical trial on the Covid-19 vaccine. The vaccine called Soberana 1 contains RBD, which is part of the spike protein, and an adjuvant that enhances the immune response. (Phase I-II combined phase).

12. On August 26, a Russian biological research center known as the Vector Institute registered a Phase I-II trial for a coronavirus vaccine they called EpiVacCorona. The vaccine contains viral peptides. (Phase I-II combined phase).

13. In addition to the mRNA vaccine, Sanofi has developed another Covid-19 vaccine based on viral protein. They produced the proteins with engineered viruses that grow inside insect cells. GSK firm supported these proteins with adjuvants that stimulate the immune system. The companies launched a Phase 1/2 clinical trial in September, with plans to start Phase 3 trials in December (Phase I-II combined phase).

14. Clover Biopharmaceuticals company has developed a vaccine containing coronavirus proteins. To further stimulate the immune system, the vaccine is supplied with adjuvants produced by British pharmaceutical manufacturer GSK and American company Dynavax.

15. The Australian company Vaxine has developed a vaccine that combines viral proteins with an immune-stimulating adjuvant. They successfully completed their Phase I trials in July and expect to start Phase 2 trials in September.

16. Medicago, based in Canada, uses a type of tobacco for vaccine production. They passed the virus genes on tobacco leaves. From there, plant cells produce protein shells that mimic the virus. In July, pharmaceutical manufacturers started Phase I trials on a plant-based Covid-19 vaccine, combining it with adjuvants produced by GSK and Dynavax.

17. A vaccine developed at the University of Queensland; Australia uses modified viral proteins to achieve a stronger immune response. Experiments on hamsters have shown that the vaccine protects them from coronavirus. In July, the University launched Phase I trials of the vaccine by combining proteins with an adjuvant made by CSL.

18. A second tobacco-based vaccine is being developed at Kentucky BioProcessing, an American subsidiary of British American Tobacco. It uses a species of tobacco called Nicotiana

benthamiana to produce viral proteins. After completing the preclinical tests in the spring, they started the Phase I trial in July.

19. Taiwan-based vaccine manufacturer Medigen has produced a vaccine that consists of a combination of spike proteins and Dynavax's adjuvant. They started Phase I trials in September.

20. Taiwan-based vaccine maker Adimmune received permission to start the Phase I trial on 20 August. The vaccine contains the RBD portion of the spike protein of the virus.

21. In July, researchers at the West China Hospital of Sichuan University published a study in the Nature journal explaining that a vaccine made from the RBD site of the spike protein could protect mice and monkeys from coronavirus. On August 4, they received approval for Phase I. They added the gene encoding the RBD region to a virus, then infected insect cells with this virus, allowing the molecule to be produced in large quantities.

22. New York-based COVAXX, a subsidiary of United Biomedical, has produced a vaccine containing various viral protein fragments. They launched phase I trials in Taiwan on September 11th.

23. In the spring, researchers at the University of Tübingen in Germany created a vaccine made of eight parts of two viral proteins, along with an immune-stimulating adjuvant. They started the phase I trial in September.

24. On July 18, North Korea's State Science and Technology Commission announced on its website that they are launching clinical trials on a vaccine based on a fragment of the coronavirus spike protein.

25. Indian company Bharat Biotech, in collaboration with the Indian Medical Research Council and the National Institute of Virology, has designed a vaccine called Covaxin based on an inactivated form of the coronavirus. The company reported that Phase I-II trials were initiated in July. (Phase I-II combined phase).

26. The central Asian nation of Kazakhstan reported that they have begun research on a vaccine made from inactivated coronaviruses over the summer. On August 28, their Research Institute for Biological Safety Problems started Phase I studies of the vaccine known as QazCovid.

Phase II:

1. **BNT162b2 vaccine:** German BioNTech company has collaborated with New York-based Pfizer and Chinese pharmaceutical manufacturer Fosun Pharma to develop an mRNA vaccine to be delivered in two doses. They started Phase I-II trials of two versions of the vaccine in May. They demonstrated that both versions elicit antibodies and T cells response to SARS-CoV-2 in volunteers. They found that the version called BNT162b2 caused significantly less fever and fatigue side effects, and they chose to move on to Phase II-III trials with this version. (Phase II-III combined phase).

2. Indian vaccine manufacturer Zydus Cadila started testing a DNA-based vaccine in July and became the second company in India to enter the Covid-19 vaccine race after Bharat Biotech. They started the Phase II trial on August 6.

3. The German company CureVac launched a Phase I trial for the mRNA vaccine in June and moved on to a Phase II trial in August. CureVac collaborated with Elon Musk's company Tesla to establish mRNA "micro-factories" that could produce billions of doses of vaccine.

4. **AZD1222 SARS-CoV-2 vaccine:** The vaccine developed by the British-Swedish company AstraZeneca and Oxford University is based on a chimpanzee adenovirus named ChAdOx1. In preliminary research on monkeys, the vaccine was found to provide protection. Vaccine developers did not detect any serious adverse effects in Phase I-II trials. The vaccine has been shown to produce antibodies against coronavirus and provide an immune response. Phase III trials of the vaccine have begun in Brazil, South Africa and the United States, as well as Phase II-III trials in the UK and India. On September 6, AstraZeneca discontinued their global trial because of a volunteer developing transverse myelitis as a side effect. Trials in the UK and Brazil were resumed on September 12. Trials are on hold in other countries. (Phase II-III combined phase).

5. In July, the Chinese company Anhui Zhifei Longcom began Phase II trials for a vaccine that is a combination of viral proteins and an immune-stimulating adjuvant. The company is part of Chongqing Zhifei Biological Products and has partnered with the Chinese Academy of Medical Sciences.

6. Researchers at the Chinese Academy of Medical Sciences Institute of Medical Biology, who had previously developed vaccines for polio and hepatitis A, launched a Phase II trial for an inactivated virus vaccine in June.

Phase III:

1. **mRNA-1273 SARS-CoV-2 vaccine:** Moderna develops vaccines based on messenger RNA (mRNA) to produce viral proteins in the body. There are no vaccines released so far. In January, they started developing a vaccine for the coronavirus. Working with the American National Institute of Health (NIH), they have shown that the vaccine they developed creates immunity in monkeys. In March, the company put the first Covid-19 vaccine into human trials, which reported promising results. The vaccine progressed to Phase III on 27 July. On September 17, Moderna shared their protocols to determine whether their vaccines were safe and effective. They reported that their work could continue until the end of 2020 or early 2021.

2. **Ad5-nCoV COVID-19 vaccine:** The Chinese company CanSino Biologics, in partnership with the Institute of Biology at the country's Military Medical Sciences Academy, has developed an adenovirus-based vaccine called Ad5. In May, they published promising results following the Phase I safety trial, and in July, in Phase II trials, they said the vaccine developed a strong immune response. The Chinese military approved the vaccine as a "specially needed drug" for a year on June 25, and trials on soldiers began. Saudi Arabia and Pakistan have also participated in Phase III trials of this vaccine.

3. **Sputnik V vaccine:** The Gamaleya Research Institute, part of the Russian Ministry of Health, started clinical trials of a vaccine called Gam-Covid-Vac in June. A combination of two adenoviruses, Ad5 and Ad26, both with a coronavirus gene attached to them, are used. On August 11, President Vladimir V. Putin announced that a Russian health care regulator had approved the vaccine, renamed Sputnik V, before Phase 3 trials had even begun. On September 4, the Gamaleya researchers published the results of the Phase I-II trials. In one small study, Sputnik has been reported to have mild side effects and produce antibodies to the coronavirus. The article published in the BMJ received negative reactions from the scientific community in terms of the small size of the study group, the absence of a placebo group, and safety risks (13). Meanwhile, Russia made agreements for supplying the vaccine to countries such as Brazil, Mexico and India.

4. **CoronaVac SARS-CoV-2 vaccine:** Sinovac Biotech, a private Chinese company, is testing the inactivated virus vaccine called CoronaVac. In June, the company announced that Phase I-

II trials of 743 volunteers had no serious side effects and the vaccine produced an immune response. Sinovac then initiated a Phase III trial in Brazil in July and Indonesia the following month.

5. **New Crown COVID-19 vaccine:** Wuhan Biological Products Institute has developed an inactivated virus vaccine. The Chinese State-owned company Sinopharm has started clinical tests of this vaccine. Phase I-II trials have shown that the vaccine induces antibody production in volunteers, causing fever and other side effects in some volunteers. They launched Phase III trials in the United Arab Emirates in July and in Peru and Morocco the following month. On September 14, the United Arab Emirates gave emergency approval for the use of Sinopharm's vaccine on healthcare workers.

6. Sinopharm is also testing a second inactivated virus vaccine developed by the Beijing Institute of Biological Products. For Phase III studies, 5,000 people in the United Arab Emirates will receive the Wuhan Institute version, while 5,000 people will take the Beijing Institute version. On September 14, the United Arab Emirates also gave emergency approval for this vaccine to be used on healthcare workers.

7. **Ad26.COV2-S (JNJ-78436735) vaccine:** Ten years ago, researchers at Beth Israel Deaconess Medical Center in Boston developed a method for making vaccines out of a virus called Adenovirus 26, or Ad26 for short. Johnson & Johnson has developed vaccines for Ebola and other diseases with Ad26 and has now made a vaccine for the coronavirus. The vaccine has been found to have a protective effect in experiments on monkeys. Johnson & Johnson initiated Phase I-II trials in July. Janssen Vaccines & Prevention BV of Johnson and Johnson announced the initiation of a large-scale, multi-country Phase III trial ENSEMBLE study for the research SARS-CoV-2 vaccine candidate, Ad26COV-S, JNJ-78436735, COVID-19 vaccine.

8. The Bacillus Calmette-Guerin vaccine was developed in the early 1900s as a protection against tuberculosis. The Murdoch Children's Research Institute in Australia is conducting a Phase III trial called BRACE to see if the vaccine partially protects against coronavirus.

While there are no vaccines approved for general use so far, some have received limited approval for use in certain populations and in certain situations. These are CanSino Biologics' Ad5-nCoV COVID-19 adenovirus vaccine, which was approved for use in the Chinese Army, Russia's Sputnik V vaccine, which was approved for early use, Sinovac Biotech's inactivated CoronaVac vaccine, and Sinopharm’s two vaccines (Wuhan and Pekin), which were approved in the United Arab Emirates (7,8,12).

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One Medicine, One Health, One World

Levent ALTINTAS

Abstract

The concept of "One Medicine, One Health, One World" is a concept that importance has been better understood in recent years and it requires interdisciplinary study. This concept suggests that human and veterinary medicine must act together to create a healthy universe. Indeed, animal diseases have caused disaster throughout human history. The source of the emergency we are experiencing nowadays is a zoonotic factor. Still, despite all these today, the concept of "One Medicine" or "One Health" is not supported sufficiently. It should not be forgotten that for a healthy person; first, a healthy animal and a healthy environment are essential. Today, it is celebrated as "One Health Day" on 3 November every year in order to raise awareness for a single health approach.

Within the scope of this presentation, the concept of "One Medicine, One Health, One World" will be summarized by referring to its historical process.

Keywords: One health, one medicine, one world, zoonosis.

Introduction

The concept of "One Medicine, One Health, One World"; it is a concept that importance has been understood better in recent years and necessarily requires interdisciplinary studies. In fact, this concept, which origin dates back to the 1800s; that have gained more importance and has come to the fore again due to especially in recent years caused by negative impact of zoonotic diseases on human, animal and environmental health as well as on the international economy and trade. Of course, in today's constantly changing the world; efforts to control especially infectious and epidemic diseases and the potential mobility of diseases among wildlife, domestic animals and humans have also been instrumental in understanding the importance of the concept of "One Medicine, One Health, One World".

"One Medicine" expression is a concept that focuses on infectious diseases, serves public health in the human-animal-environment triangle and at the same time supports an interdisciplinary approach against zoonotic diseases; and this concept is qualified in developed countries, "One health concept" together with human, animal and environmental health.

The concept of "One Health" is a comprehensive concept that embraces doctors, veterinarians and other healthcare professionals working in both human and animal health fields, and it is a concept that focuses on the control and spread of zoonotic diseases that pose a threat to public health.

The concept of "One World" refers to the cooperation of related disciplines in order to reach optimal conditions of human, animal and environmental health, starting from local applications, respectively, on a national, international and ultimately universal scale; and as a result of this partnership, it aims to protect World Health.

Today, approximately 60% of the diseases seen in humans are in zoonotic character; while the number of these diseases was 86 in the 1950s and around 150 in the 1970s, it reached 200 in the 2000s and new ones have been added to this number every day. Approximately 75% of new diseases in humans, or in other words, 3 out of 4 new diseases are from animal origin. More than 75% of the diseases encountered in humans, especially in the last thirty years, are new or re-regaining importance zoonotic diseases (such as Covid-19, Rabies, SARS, Ebola Hemorrhagic Fever, Bird Flu, West Nile Virus, Monkey Flower, Tuberculosis). Today, every 10-15 minutes a person dies from rabies and a total of around 2.7 million people each year from zoonotic diseases; also, food-borne infections are largely (more than 90%) caused by animal origin foods and 80% of the agents used as potential bioterrorist agents today are composed of zoonotic pathogens.

In fact, animal diseases have always been the most important source of trouble that caused great disasters throughout human history in every period. For example; because of the plague epidemic in Europe in the 17th century, European Union countries have almost reached the point of disappearing from the map; with the epidemic that developed due to the Cattle Plague disease in the 18th century, agriculture and the economy became inoperable. It should not be forgotten that there is a zoonotic factor at the root of the pandemic-induced extraordinary situation we are experiencing these days. At the same time, the borders of the country do not constitute an obstacle for the vectors, reservoirs or microorganisms that play a role in the spread of the diseases seen today. Additionally, only human cases are inadequate in the eradication of the disease in the emerging epidemic diseases, and only 3% of the reservoirs are people among the known zoonotic diseases. Therefore, for humans to be healthy, it is essential that animals to be healthy.

To summarize this information and the latest developments “One Medicine/One Health Concept” that foundations date back to the 1800s has come to the fore again today and its importance has been understood once again. At this point, while the expression “One Health” is an approach that focuses on communicable diseases and looks at public health through the triangle of human, animal and environment; on the other hand, “One Health Concept” can be defined as a concept that aims to raise awareness in the society in the context of disease epidemiology and thus create preventive strategies in order to improve the quality of public health. For this reason, it is celebrated as “One Health Day” every year on November 3 in order to explain the single health approach to the whole world and to raise awareness. The effectiveness of One Health approach will be provided by a much sectoral establishment between institutions dealing with especially veterinary services and public health services and also social science, environment and wildlife.

History

Although the concept of single health is actually accepted as a new term, its foundations are very old. The effects of environmental factors on human health and the hypothesis that public health will depend on a clean environment; it is even mentioned in texts named “In the Air, in the Water and on the Ground” written by the Greek doctor Hippocrates.

In the 1700s, the Italian doctor Dr.Med.Vet. Epidemiologist Giovanni Maria Lancisi; investigated the role of the physical environment in the spread of diseases in humans and animals. Lancisi is also one of the first advocates of the necessity of using mosquito nets to dry marshes in order to prevent malaria in humans, as well as the pioneers of researchers working on rinderpest control in cattle. Following Lancisi, Claude Bourgelat established the first

Veterinary Faculty in France in the 1700s and a formal education system for interactions between human and animal health was put into practice in Europe. With the French Revolution realized in these periods; the idea that human, animal and environmental health are interconnected has been revived, and the field of public health expertise has been brought to the agenda by Alexandre Parent-Duchâtelet and Louis-René Villerme, and the same scientists have contributed to the development of veterinary expertise in public health field.

German physician and pathologist Rudolf Virchow, who is accepted as one of the founders of the concept of comparative medicine and One Health; he coined the term "Zoonosis" in the late 19th century and he pioneered the concept of "One Medicine/One Health" with the expressions of: "*Between animal and human medicine there is no dividing line - nor should there be. The object is different but the experience obtained constitutes the basis of all medicine*". According to Virchow, there is no clear line defining the boundaries between human medicine and veterinary medicine, or even such a line should not exist. This understanding, which can also be described as "One Physician = One Medicine = One Health", has been supported by many scientists. This view is developed as "*Veterinary Medicine and Human Medicine complement each other and this concept should be perceived as the One Medicine Concept*" by one of Virchow's students, a Canadian human doctor and advocate of comparative medicine Sir William Osler. The phrase "One Medicine" is credited to Sir William Osler, a disciple of Virchow's and the "father" of veterinary pathology in North America, and is a philosophy that has been championed by W. Calvin Schwabe.

In the twenty-first century, W. Calvin Schwabe, a Veterinary Epidemiologist, proposed that the Human Medicine/Veterinary Medicine joint approach to zoonotic diseases should be expressed as "One Medicine"; and in 2010, with a joint declaration published by WHO, FAO and OIE "One Health" concept has been accepted as an approach that can be achieved through interdisciplinary cooperation.

Situation in TURKEY

In our country, Zoonosis National Committee was established in 1991 as a part of the One Health approach. The committee changed its name to Zoonotic Diseases National Committee in 2018 and which accomplished various activities (protocols, subcommittees and Turkey Zoonotic Diseases Action Plan such as the establishment) in Turkey.

Based on the context of "One Medicine, One Health, One World", with a joint declaration made by the TVHB Central Council and TTB Central Council in 2009, the importance of the "One World-One Health" approach was emphasized and the understanding of acting together on common issues such as zoonotic diseases was revealed. In 2014, with the Headquarters of Food and Control under the Ministry of Food, Agriculture and Farming as it was then known, dated 20.01.2014 and numbered 71037622-010.06.02-2663 on "Fighting Animal Diseases and Animal Movement Control" attention was drawn to the concept of "One Health" and information was given about the duties and responsibilities of veterinarians in achieving success in this concept. In this context, animal health, animal welfare, public health and food safety issues were especially emphasized. At the same time, TVHB and EKMUD, with the participation of relevant ministries, organized Zoonotic Diseases Symposium, and related to zoonotic diseases congress, workshops and other activities time to time.

Stakeholders

Among the stakeholders who can take part in "One Medicine, One Health Concept (doctors, veterinarians, epidemiologists, public health experts, bioinformatics, biologists, forest-fisheries and agricultural engineers and of course politicians) should take part be included. The approach to this concept is that human and veterinary medicine act together. In this context, close cooperation is expected between doctors, veterinarians, dentists, nurses, other healthcare professionals and environmental disciplines. Thus, starting from the local, it is aimed to protect the World Health with the cooperation of the relevant disciplines in order to create optimal conditions for human, animal and environmental health, first nationally, then internationally and ultimately globally (Fig 1).

To reach this goal; integration of Medical, Veterinary Medicine and Public Health Education institutions in education should be provided; professional publications, conferences, workshops on the subject should be held, and communication among health organizations should be developed; researches should be done on disease transmission between species; cooperation between sectors should be established in the development and evaluation of new diagnostic methods, drugs and vaccines for the control and prevention of interspecies diseases.

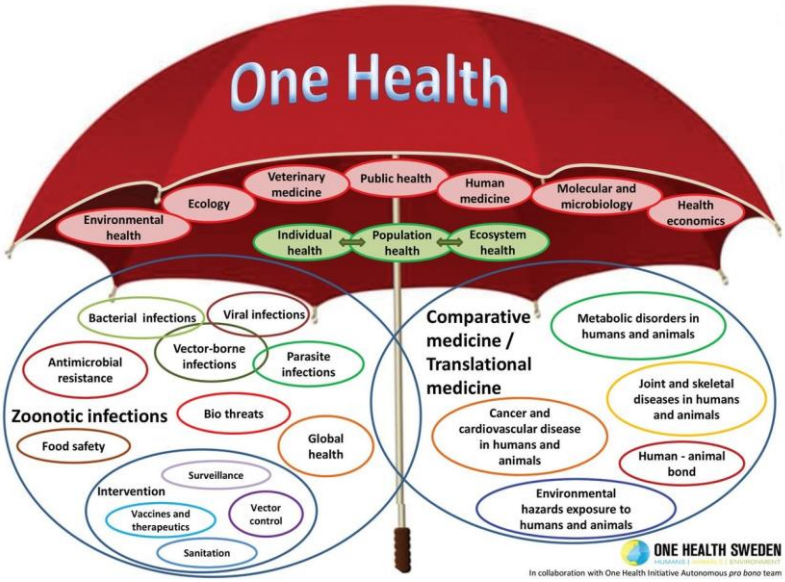

Figure 1. The One Health Umbrella.

Animal and human health necessities should be improved with the cooperation among doctors, veterinarians and all other health sciences. The cooperation among veterinary medicine, human medicine and public health schools should be developed, and education and research centers should be established, especially in public places. Of course, one of the most important problems that can be encountered while ensuring this cooperation is the legislative problems that may arise. At this point, politicians from the profession groups we mentioned at the beginning of the subject should step in and contribute to the process at the point of preparing legal regulations on the subject and putting them into effect as soon as possible.

Conclusion

Worldwide health safety, which we call “One World”, is a task that is under the common responsibility of all countries sharing the earth, and ensuring ideal community health. Providing this situation is possible if the relationship between humans and animals is carried out in a regular and controlled manner and a strong cooperation among relevant institutions is ensured. Considering the fact that humans and animals live together; in order to protect global health first locally, then nationally and internationally, it is inevitable for medical doctors and veterinarians to come together and act together, which means the concept of “One Medicine-One Health”. In fact, it is possible to protect the whole world in a way by protecting animal health. In this way, human health will also be protected. Because, protecting the animal and human health, means protecting the whole world.

It should not be forgotten that, a healthy person and a healthy society would only be possible with a healthy animal and a healthy environment.

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CORONAVIRUSES - COVID 19

Metin DOĞAN

Coronaviruses (CoVs) were first isolated in 1965 and named 'corona' due to their crown-like appearance on their surface under electron microscope. The CoVs belong to the order *Nidovirales*, family *Coronaviridae*, and the subfamily *Coronavirinae*. They are enveloped, single stranded, positive polarity RNA viruses and microorganisms with genomes of 26 to 32 kilobase.

There are four subgroups named alpha coronavirus (αCoV), beta coronavirus (βCoV), delta coronavirus (δCoV) and gamma coronavirus (γCoV). The αCoV and βCoV subgroups are mostly of bat and rodent origin and generally cause respiratory diseases in humans and gastroenteritis cases in animals. In δCoV and γCoVs, the source is mostly birds and this group has not been reported to infect humans. After the virus was isolated from birds, the virus was also isolated from animals such as bats, camels, cats, dogs, pigs and whales.

These viruses can cause respiratory, enteric, hepatic and neurological diseases in a variety of hosts.However, the clinic in humans, is often obscure. SARS-CoV and MERS-CoV are examples that can cause severe clinical pictures in humans. SARS-CoV-2, which was detected in Wuhan in December 2019, is the seventh CoV species that can cause infection in humans, and it is accepted as the Sarbecovirus subgenus of the βCoV genus in taxonomy.

In December 2019, pneumonia cases of unknown cause were detected in Wuhan, China and spread to the whole world in a short time. The causative agent of this epidemic has been identified as a new coronavirus and has been named SARS-CoV-2 due to its similarity to Severe Acute Respiratory Syndrome-Coronavirus (SARS-CoV). World Health Organization (WHO) defined the name of the disease as COVID-19 (Corona Virus Disease 2019).On January 30, 2020, WHO declared the outbreak as a global emergency. The first case in our country was reported on March 11, 2020.

It was revealed that SARS-CoV-2 infected cases are clustered in the local seafood market place in Whan. The identification of many SARS-like CoVs in bats strengthens the idea that these animals host the virus as a natural reservoir. In particular, SARS-CoV-2 is highly associated with bat CoVs and shows 100% amino acid similarity to bat SARS-like coronavirus SL-CoVZC45 in nsp7 and E proteins. Therefore, these data suggest that bats are also possible viral reservoirs for SARS-CoV-2. SARS-CoV-2 showed the highest genomic match with 96.2% identity to a bat CoV RaTG13. Therefore, the virus is thought to be transmitted from bats.

Identifying the morphological characteristics of coronaviruses is important in terms of pathogenesis. Pointed surface protein, Spike (S) protein; The membrane (M) protein and the envelope (E) together with the protein are embedded in the lipid double layer structure. This double-layered lipid layer is produced from the host cell membrane and surrounds the Nucleocapsid (N) protein and its RNA. Unlike other RNA viruses, coronaviruses have a very large virion as size. Although the genome of coronaviruses contains a variable number (6-11) ORF (Open Reading Frame), the SARS-CoV-2 genome contains 12 different ORFs. Essentially two-thirds of the viral RNA is present in the first ORF. ORF1a / b; encodes two large polyproteins. These are polyprotein1a (pp1a) and polyprotein 1ab (pp1ab). Then pp1a and pp1ab transform into 16 nonstructural proteins (NSP). These NSPs are important proteins involved in the transcription and replication of the virus. The remaining ORFs of the virus genome encode four major structural proteins including S glycoprotein, E protein, M protein

and N protein, and some accessory proteins. The main structural proteins of SARS-CoV-2 are S, E, M and N proteins. Their genomes also encode non-structural NSPs. Unlike other coronaviruses, SARS-CoV-2 lacks hemagglutininesterase.

S protein is located on the surface and it is a protruding protein that gives the virus its name. It is responsible for host cell tropism and is responsible for attachment and entry into the cell. It has S1 and S2 subunits, S1 functions in the attachment to the host cell and S2 functions in fusion with the cell membrane and entry into the host. Since the S protein is the first point of contact with host receptors, it can be a good therapeutic target to prevent the virus from binding to the receptors and viral entry into host cells.

M protein can be in two forms, long form and compact form, and it is the most common protein in the coronavirus. M protein translation takes place in polysomes fused to the endoplasmic reticulum membrane and then interacts with E proteins to form the virion. It shapes the nucleocapsid and thus the viron. In addition, envelope formation and budding play a role. The M protein is known to inhibit Nuclear Factor Kappa B, which helps in the immune response, and reduce cyclooxygenase-2 levels, thus increasing the proliferation of the viral pathogen. It has also been shown to induce apoptosis in the cell and lead to the activation of beta interferons (IFN-β).

E protein is a protein made up of an average of 75 amino acids in both monomeric and homo-pentameric form, relatively small in size. While this protein is not required for coronavirus genome replication, it affects virus morphogenesis, budding, assembly, intracellular interaction, and virulence. E protein is highly immunogenic. Modification or deletion of different motifs within the E protein has weakened the virus and it has been promising for vaccine studies.

N protein is an RNA binding protein required for viral RNA transcription and replication. Its main function is to bind to the viral RNA genome and package as a helical nucleocapsid or RNP complex. However, it has several important roles, such as the creation of helical ribonucleoproteins (RNP) during packaging of the viral RNA genome, regulation of viral RNA synthesis in replication / transcription, and modulation of infected cell metabolism. In addition, N protein is also a regulator of host-pathogen interactions such as actin reorganization, progression of the host cell cycle and apoptosis. It has a highly immunogenic structure that can induce a protective immune response.

Non-structural proteins (NSPs) are numbered from 1 to 16. The function of some of these proteins in the cell has not been clearly established. These proteins are mainly involved in the viral replication-transcription complex.

NSP1 is also known as the leader protein. It is known to have a function of suppressing gene expression in the host cell. NSP2 functions by binding to prohibiting protein 1 and 2. It plays a role in cell cycle progression, cell migration, cellular differentiation, apoptosis, and mitochondrial biogenesis. NSP3; It is known as papain-like proteinase. It is the largest protein in SARS-CoV-2 with the exception of polyprotein1a / 1ab. This protein is responsible for the release of NSP1, NSP2, and NSP3 from the N-terminal region of polyprotein 1a and 1ab. It is a suitable target for antiviral strategies. NSP4; It has a role in viral replication and membrane rearrangement. NSP12 is RNA-dependent RNA polymerase that replicates viral RNA. For this function of NSP12, it must complex with an NSP7-NSP8 heterodimer and a NSP8 monomer. NSP12 alone shows poor processability in RNA synthesis. NSP13 is a helicase enzyme. It has been shown that the binding of NSP12 with NSP13 can increase the helicase activity of NSP13. It also plays a role in the translation and stabilization of mRNA. NSP14 is known to have 3′-5 exoribonuclease activity and N7-methyl transferase activity in coronaviruses. NSP15 has been

characterized biochemically as an endoribonuclease. It specifically targets and disrupts viral polyuridine sequences to prevent the host immune sensing system from detecting the virus. It has also been suggested that it disrupts the viral dsRNA to prevent the virus from being recognized by the host.

In pathogenesis, SARS-CoV-2 has a cellular entry mechanism similar to SARS-CoV. The coronavirus S protein plays a key role in virus attachment and entry into target cells. In addition, trans-membrane protein serine protease 2 (TMPRSS2) from host cell proteases and other entry factors into cell also play a role in this mechanism. The SARS-CoV-2 cell entry receptor is angiotensin converting enzyme 2 (ACE-2). While it interacts with the S protein and ACE-2 receptor on entry into the cell, the S1 and S2 subunits have separate roles. While the S1 subunit is involved in receptor attachment, the S2 subunit contains a fusion peptide and transmembrane unit to trigger fusion of the cell membrane with the virus. Therefore, in order for fusion to occur, the S protein must be divided into 2 regions in the cell membrane. This structural separation is called cleavage. This separation depends on the proteases in the host cell.This is demonstrated by the cellular serine protease TMPRSS2, furin, endosomal cathepsin B and L activities. Therefore, the occurrence of infection depends on the proteases and protease cleavage sites found in different cell types. In addition, there is a widespread tissue expression of ACE-2, the entry receptor of the virus. Therefore, extrapulmonary spread can also be seen.

In the replication cycle of the virus, the translation of the positive polarity RNA Polyprotein1a / 1ab by the host cell ribosomes is the first step. In this step, 16 NSPs that will form the replication-transcription complex and do not participate in the viral structure are coded. The next step is a synthesis of nested subgenomic RNA. This synthesis proceeds in a negative direction and the production of subgenomic mRNAs and genomic RNA encoding structural proteins is completed.The management and termination of the synthesis is provided by the regulatory sequences between the ORFs. With the translation of subgenomic RNAs, S, M and E proteins are produced and arrayed on the endoplasmic reticulum membrane. After the N protein is produced, it wraps the genomic RNA and the nucleocapsid is formed. As all these components produced move from the endoplasmic reticulum to a Golgi vesicle, the assembly phase is completed and a whole viral particle called the virion is formed. The viral particle formed is released from the host cell with exocytosis by budding.

Effects of infection; SARS-CoV-2 mostly causes cytopathic changes by infecting enterocytes and pneumocytes. Type 2 pneumocytes, and bronchial epithelium that have lost their cilia are the leading targets. Infection manifests itself with mild or severe respiratory tract involvement after an incubation period of 1-14 days (25). Pneumonia, is mostly bilateral, which can sometimes be unilateral, can lead to respiratory failure and death.

Respiratory failure is often associated with hyperinflammation. It has been reported that the cause of hyperinflammation may be cytokine storm syndrome. Mediators such as interleukin 6, interleukin 8, E-cadherin, MCP-1, VEGF are involved in this mechanism. Although it mainly affects the lungs, patients with multi-organ dysfunction have also been reported, including the heart, liver, kidneys, blood vessels, and other organs. SARS-CoV-2 causes silium dysfunction and cytopathic effects in respiratory epithelial cells.

It is believed that both cellular and humoral immune mechanisms play a role in this pathogenesis. In addition to the direct macrophage and lymphocyte stimulated response, T cell and B cell mediated immune mechanisms are initiated against SAR-CoV-2. This process includes SARS-CoV-2 antigen presentation and activation of B cells. SARS-CoV-2 attacks epithelial cells of the respiratory mucosa and spreads to other cells, infecting peripheral white blood cells and immune cells, especially T lymphocytes. This partially explains the

lymphopenia in the clinic of COVID-19 patients. Damage to lymphocytes, including T lymphocytes, by the coronavirus leads to the predisposition to secondary bacterial infections and the exacerbation of the disease. Increase in proinflammatory cytokine levels and decrease in anti-inflammatory cytokines are indicators of T cell mediated response to SARS-CoV-2. It has been reported that this may cause to hyperinflammation which causing to severe pneumonia, and to cytokine storm.

Among the biochemical tests, the increase in C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), lactatedehydegenase (LDH) and D-dimer values and low serum albumin and hemoglobin levels are important indicators of COVID-19. Increased white blood cell count, increased neutrophil count, decreased lymphocytes, decreased albumin concentration, increased lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, creatinine, cardiac troponin, prothrombin time procalcitonin levels are seen in most patients.

The decrease in immunoglobulin levels in COVID-19 can be explained by its effects on the antibody-producing cells, B lymphocytes. Even if the immunogen components of SARS-CoV-2 stimulate antibody production, immunoglobulin levels may remain low. Antibodies developed against the S protein of SARS-CoV-2 may be responsible for infecting immune system cells. Antibody-dependent enhancement mechanism (ADE), which requires prior exposure to similar antigenic epitopes of coronaviruses; It may be responsible for other observed severe illnesses, including acute respiratory injury (ARDS), cellular immunodeficiency, coagulation activation, myocardial damage, liver and kidney damage, and secondary bacterial infection. It is presumed the effect of ADEto be an important pathogenesis mechanism resulting in persistent inflammation, lymphopenia and cytokine storm in severe cases. However, although it is believed that the cytokine storm is responsible for this pathogenesis, the true pathogenic mechanisms have not yet been elucidated.

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THE EFFECT of VITAMIN D SUPPLEMENTATION on VIRAL INFECTIONS

Seçil Özkan[1] Alper Karagöz[2] Funda Karabağ Çoban[2]

ABSTRACT

Viral infections are among the necessary causes of mortality that may spread quickly and cause epidemics [1]. The best defense in viral infections is our body's immune system. With a balanced and adequate diet, immune functions work correctly and strongly. So as to produce this situation, the mandatory macro and micro nutrients should be sufficient. Vitamins and minerals, particularly vitamins such as A, C, D and E, and minerals such as copper, zinc, iron and selenium have been reported to play an vital role in maintaining a healthy immune response [2- 5]. In this review, we discuss the impact of vitamin D supplementation and viral infections.

INTRODUCTION

Outbreaks of Severe Acute Respiratory Syndrome and Middle East Respiratory Syndrome in 2002 discovered that coronaviruses are also pathogens that may cause fatal respiratory diseases in humans [6]. Covid-19 virus, which apperared in China at the end of 2019 and unfold everywhere the planet and become a pandemic, was additionally shown to be caused by a new type of coronavirus distantly relating to SARS coronavirus. Currently, it is known that there's no drug that may undoubtedly stop or treat the Covid-19 outbreak [7- 9].

Various viruses widespread flu or upper respiratory tract viral illness are the common contagious illness, with quite two hundred viruses conduce to the some indication. Circumstantial argument primarily indicate to the investigation that winter low standarts of vitamin D were connected to increase in contagious illness. Many other research as find out a connection among lack of vitamin D, elevated risk of breathe illness. For example, in a study from October 1988 between 1994 investigation knowledge on rating vitamin D standarts and upper respiratory infections in a population of almost nineteen thousand adolescents and adults. Participants with the least vitamin D standarts were thirty six percent more probably to certify having a present upper respiratory viral illness than those with peak stndarts [10]. Some metaanalysis and systematic investigation are available previous studies demolition that vitamin D reinforcement may prevent acute respiratory viral illness [11].

Also, vitamin D helps calcium to be absorbed, transported and stored in bones [12]. Other its roles in maintenance of bone integrity and calcium homeostasis, it therefore warns the growing of immune cells. The low mortality rate in Northern European countries has been associated with the widespread use of vitamin D supplements and the low frequency of deficiency. The high mortality at latitudes above 35 ° in the Northern Hemisphere suggests that the situation may be associated with vitamin D deficiency [13, 14].

While, In the correlation between Covid-19 and vitamin, the impact of calciferol on the T cell (Th) response, especially in the case of viral or bacterial infection, the immune system responds by secreting anti-inflammatory and pro-inflammatory cytokines. The cytokine storm caused by the excessive secretion of these cytokines is connected with the severity of Covid-19 and is shown as an important cause of Covid-19 related mortality [15].

Vitamin D decreases helper Th1 response and increases Th2 and regulatory Th response. Thus, whereas the release of proinflammatory cytokines decreases. The release of anti-inflammatory cytokines is increasing. It has been reportable that with this regulation effect of vitamin D on

the immune system, it may prevent cytokine storm and consequently acute respiratory distress syndrome [16, 17]. As another mechanism, it has been suggested that vitamin D may decrease the severity of Covid-19 by rising angiotensin converting enzyme 2 (ACE-2) expression and decreasing pulmonary vasoconstriction [18]. Many randomized controlled clinical studies have been conducted to investigate the protection of vitamin D supplementation against the risk of acute respiratory infections. In a meta-analysis including a total of 25 randomized controlled clinical trials, vitamin D supplementation was found to reduce the risk of acute respiratory. It has been reported that the protective effect is significant in those who receive vitamin D supplements on a daily or weekly basis, and there is no significant effect in terms of protection in those who use it as a bolus dose. [19]. Various surveys have day experimental cited the safety of calciferol reinforcement at doses of up to one hundred micrograms per with a diem 21 more investigation committing a tolerable upper limit of two hundred and fifty micrograms per diem [20].

CONCLUSIONS

Vitamin D is believed to support the immunity system, increase cytokine release and have a more protective effect in viral infections due to daily vitamin D doses. However, a protective impact of calciferol reinforcement on pneumonia was not observed in three extra circumtance control researches, nevertheless the explication of these conclusions should take into account the pre-existing defiency of vitamin D. Also, beneficial of vitamin D in an developed phase of serious diseases is contentious, as far as many researches indicate no helpfl when apply late in crucial diseases [21, 22].

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STEM CELL TREATMENT STRATEGIES IN THE TREATMENT OF NEWBORN BRONCOPULMONARY DYSPLASIA

Selçuk GÜREL

Abstract

Bronchopulmonary dysplasia (BPD) significantly increases the morbidity and mortality of premature babies, and most therapeutic approaches for BPD are inherently preventive or supportive. Advances in neonatal medicine have resulted in an increase in the incidence of BPD, leading to an increase in the survival rate of infants born at viability limits. BPD is a chronic lung disease of premature babies characterized by arrest of alveolarization, fibroblast activation and inflammation. BPD causes significant morbidity and mortality in the neonatal period and is one of the leading causes of chronic lung disease in children. Current research into the pathogenesis of this disease has highlighted the central role of inflammation, which contributes significantly to the disease development. Cell-based therapies may represent the next groundbreaking therapy for the treatment of BPD, but there are also barriers to implementation, as well as gaps in knowledge of the role of endogenous Mesenchymal stem cells (MSCs) in the pathogenesis of BPD. MSCs have anti-inflammatory properties and have been shown to contribute to tissue regeneration in a variety of clinical settings. MSCs are particularly interesting due to their ease of isolation, low immunogenicity, and anti-inflammatory and restorative properties. Simultaneous high-quality basic science, translation, and clinical studies investigating the underlying pathophysiology of BPD, the therapeutic mechanisms of exogenous MSCs, and the logistics of translating cellular therapies will be important areas of future research.

Keywords: Bronchopulmonary dysplasia (BPD), Newborn, Mesenchymal stem cell (MSC), Cellular therapy

Introduction

Around the World, nearly one out of ten births are preterm, meaning more than 15 million babies premature. Maternal chorioamnionitis or preeclampsia induced lung injury, mechanical ventilation, hyperoxia or inflammation could lead to bronchopulmonary dysplasia (BPD), a commonly seen complication in preterm newborns. Patients with BPD experience a cessation in alveolar and microvascular development, and with aging, they are more likely to encounter asthma and early onset emphysema (1). Unfortunately, since there is no treatment for BPD, it is of the utmost importance to understand the development of alveoli, how they are repaired and regenerated after an injury to be able to develop treatments (2).

An attention must be paid to (or one must pay attention to) lung development before understanding the pathophysiology of BPD. Even though we are familiar with the morphogenesis of the lung, many studies are being conducted on cellular communications that mediate cellular growth, migration, and differentiation of lung cells. Fibroblast growth factor (FGF), transforming growth factor-ß (TGF-ß), bone morphogenetic protein (BMP), Sonic Hedgehog protein (SHH), Wnt/Beta catenin pathway, vascular endothelial growth factor (VEGF) and retinoic acid signaling pathways are shown to play a crucial role in early lung development(3). VEGF, expressed by type II pneumocytes in response to Hypoxia induced factor (HIF), favors the microvascular and alveolar development in lungs. Furthermore, VEGF have a significant role in BPD considering that the patients with BPD slightly express or do not express VEGF receptors in pulmonary endothelium. The studies have shown that platelet

derived growth factor (PDGF) and FGF induce the myofibroblasts to differentiate and are necessary elements in initiating the secondary alveolar septation (4). It is also hypothesized that WNT, BMP and TGF-ß signaling components might have a role in fibroblast differentiation during alveolarization. Moreover, myofibroblast deposition of extracellular matrix proteins like elastin and collagen, is important in secondary septation. These factors and other ECM components, act as a skeleton and provide conduit for growth factors to operate cellular growth (5).

Current Overview about Perinatal Risk Factors

BPD is a functional diagnosis; it is difficult to understand exactly which exposure is the most harmful to the lungs because the premature babies are exposed to many stress factors perinatally. Since we do not see BPD in every preterm baby and despite the novel treatment methods in newborns, we observe an increase in BPD rates, which leads us to think BPD has a multifactorial etiology which might affect either prenatal or postnatal lung development. The risk factors determined by statistical correlation are preterm birth, maternal smoking and socioeconomical history (6).

Preeclampsia and chorioamnionitis related intrauterine growth restriction are associated with threefold increase in BPD among the babies that were born before 29thgestational week. It also triggers the secretion of cytokines and growth factors that inhibit the development of fetal lung microvasculature and alveoli. Placental abnormalities like gestational hypertension, preeclampsia and eclampsia are among the antenatal risk factors for BPD (7). After the birth, invasive mechanic ventilation and supplementary oxygen induced lung injury or sepsis related inflammation are also associated with BPD. Due to their immature lung anatomy and apneic respirations, preterm newborns are exposed to hypoxia, which like hyperoxia, deteriorates the alveolar and microvascular development (8).

BPD up-to-date treatment methods

The most direct approach in treatment is to prevent the aggressive, long-term invasive ventilation need. Antenatal maternal corticosteroid therapy and prophylactic artificial surfactant administration within 2 hours postpartum is still the treatment of choice to prevent respiratory distress syndrome (RDS). The use of single or multiple doses of intramuscular betamethasone or dexamethasone injection to the mother1 – 7 days before birth increases the survival rate among newborns and decrease the probability and severity of RDS. The risk of BPD is highest among babieswhose lungs are in transition from canalicular stage to the saccular stage (9).

Stem cells in BPD pathophysiology and treatment

Stem cells possess extraordinary properties compared to somatic cells. They retain their ability to regenerate and they could also differentiate into various type of cells. These two types of cells are formed via symmetric and asymmetric division of cells. Two identical, stem cells are produced via symmetric division, whereas in asymmetrical division, one stem cell and another daughter cell that is destined to differentiate is produced (11). Mesenchymal stem cells (MSC) are fibroblast-like cells that potentiate hematopoiesis; in literature, they are defined as potent repair cells that have anti-inflammatory, immunomodulatory, angiogenetic properties. Furthermore, MSC could be isolated from variety of tissue types including adipose tissue, bone marrow, lung, placenta, and umbilical cord (12).

Secretome is the collection of substances that are secreted by a cell to extracellular space. It may include but not confined to proteins, lipids, nucleic acids, and extracellular vesicles. It is

shown that many beneficial effects of stem cells including MSCs are attributed to secreted exosomes. Paracrine mediated actions seem to be responsible for the therapeutic effects of MSC in BPD models (13). These cells are large (MSCs are typically 18 μm or larger), they can obstruct small capillary flow and increase the risk of embolism. On the other hand, microvesicles like exosomes are able to circulate through the small capillaries (14). It was proven that intrathecal or intravenous administration of bone marrow derived MSCs to the mice and rats that were exposed to hyperoxia, could prevent the inflammation, alveolar simplification, vascular reduction and pulmonary hypertension while increasing their survival (15).

Latest studies have shown that in vitro 21% or 60% oxygen exposure to the human fetal lung MSCs (16-18 weeks of gestation) disrupts their function. After the MSCs are exposed to extrauterine oxygen concentrations, a change in their surface marker profile, excessive proliferation, decrease in colony forming ability and reversal of secretome profile have been observed. Conversely, human umbilical cord derived MSCs preserved their lung protective secretome profile despite their exposure to hyperoxia in vitro (16). Another advantage of MSCs is their easy and painless accessibility from sources like umbilical cord (17).

Various type of lung epithelial stem/progenitor cells are established. Multipotent MSCs and endothelial colony forming cells (ECFC)(10) are heterogenous progenitor cells in the developing lung where the microvasculature is of great importance, besides, resident lung MSCs regulate the formation, regeneration, and tissue maintenance of the alveolar microvasculature (18).

Therapeutic effects of MSCs are not limited to paracrine factors. Preserving the cells during stem cell therapy creates a connection between exogenous MSCs and injured lung which allows the cellular detection and response to extracellular environment. As MSCs are known to play a role in organelle transfer, direct cell to cell interaction might be necessary for selected therapies. In a study using lung-injured mouse model, intratracheally administered MSCs formed microtubules to transfer mitochondria to alveolar epithelium. These transferred mitochondria and subsequent increase in adenosine triphosphate levels in mouse model were thought to be the mechanism preventing lung injury (19).

During pseudoglandular stage of lung development, early Tbx4 + multipotent MSCs give rise toa variety of mesenchymal cells includingairway and vascular smooth muscle and early fibroblast-like cells (20). PDGFRα + L-MSCspromotethe lung epithelial progenitor cells that cannot form colonies, in their absence, where myofibroblasts are still undifferentiated, lung epithelial development is favored. Disrupted L-MSCs are actively involved in BPD pathogenesis. Presence of L-MSCs in tracheal aspirations from ventilated preterm babies may prevent the later development of BPD**.** Exosomes, extracellular vesicles that embodyprotein, RNA and mitochondria cocktail, are secreted by different types of cells and presumably have an active role in the paracrine therapeutic effectsof MSCs. The effect is thought to be paracrine since the MSC conditioned media injection acts through alternative macrophage activation (M2) (21).

Vascular endothelial growth factor (VEGF) is a potent angiogenic protein necessary for pulmonary vascular growth and subsequent alveolarization. VEGF is described as a component of MSC conditioned media and especially of exosomes (20). TNFα stimulated gene-6 (TSG6) protein is an anti-inflammatory molecule seenin MSC secretome and exosomes. In a study conducted on murine hyperoxia BPD model, umbilical cord derived MSC exosomes decreased the severity of lung injury and pulmonary hypertension, and TSG-6 protein is a fundamental exosomal factor for this effect (22).

Macrophage phenotype modulation could be the key mechanism that MSCs and their products decrease the lung injury**.** Long term functional results like hyperoxia related pulmonary hypertension andpulmonary function tests have shown improvement one month after exosomal injection. Experiments shown that MSC exosomes resolve the inflammation by inducing the modulation of macrophage phenotypes from M1 to M2. Surprisingly, the anti-inflammatory effects of the TSG-6 protein also caused a shift of macrophages from M1 to M2 phenotype (23).

Phase 1-2 Applications – Results

Rejection is an expected adverse effect seen allogenic cell treatments (24). In 2014, nine babies that were born 23 – 29 weeks who needed mechanical ventilation were given intra tracheal MSC. Even though it is a feasible method and no side effects were reported, its effects on BPD were not mentioned in the study. At two-year follow-up, no respiratory and neurological complications were noted (25).

Two babies that were born in 24thgestational week, patients with BPD and severe pulmonary hypertension who are dependent on aggressive ventilation were treated with multi dose intravenous MSCs when they were 85 day and 5-month-old**.**Following MSC administration, a decrease in inflammatory cytokine and endogenous VEGF antagonist mRNA levels were seen. The authors suggested that repeated MSC administration is an applicable method without toxicity and might be used to treat BPD in early course (26).

Finally, MSC derived treatments are known to ameliorate the complications in animal models of sepsis, traumatic brain injury and in prematurity related comorbidities, and their use on other areas are still being investigated. The promising potential of stem cell derived therapies is likely due to integration of various properties (anti-inflammatory, antiapoptotic, antifibrotic, proangiogenic (27-28).

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Current Developmets in Diagnostic and Molecular Imaging

Senem ŞENTÜRK GÜÇEL

Abstract

Diagnostic and molecular imaging

Over the last 60 years, many new imaging modalities including radionuclide imaging, ultrasonography, computed tomography (CT), magnetic resonance imaging (MRI) and digital radiography have been developed. Moreover image processing techniques, computer-aided diagnosis (CAD), image recording and storage, and image transmission, most of which are included in a picture archiving and communication system (PACS,) have also been improved.

The next major area for research is molecular imaging, which will provide more fundamental and important molecular, biologic, and biochemical information in addition to anatomic information. Molecular imaging will be critical for earlier detection and management of cancer and many other genetic and metabolic diseases in the future.

Current Developmets in Diagnostic and Molecular Imaging

A. Diagnostic Imaging

W C Roentgen discovered x-ray in 1895, and H Becquerel discovered radioactivity in 1896, thereafter medical imaging has contributed significantly to progress in medicine. Over the last 60 years, many new imaging modalities including radionuclide imaging, ultrasonography, computed tomography (CT), magnetic resonance imaging (MRI) and digital radiography have been developed. Moreover image processing techniques, computer-aided diagnosis (CAD), image recording and storage, and image transmission, most of which are included in a picture archiving and communication system (PACS,) have also been improved (1).

After medical images have been produced by various modalities, they are presented to a radiologist for interpretation and a subsequent diagnosis as to the medical condition of a patient. Recently image recording and storage, and image transmission are performed by a picture archiving and communication system (PACS) in many institutions.

Diagnostic medical imaging has become established as having an important role in patient management, and especially radiologic diagnosis. Thus, the most important processes in diagnostic radiology is image interpretation and decision making. With a specialized medical knowledge and experience the radiologist leads the diagnosis as the result of a decision-making process. When the image quality is increased, the diagnostic performance of the radiologist gets better. For assisting radiologists' image interpretation, computerized analysis of medical images has recently been implemented clinically for detection of abnormalities such as breast lesions in mammograms or nodules in chest computed tomography; this is generally known as computer-aided diagnosis (CAD) (1).

An analogue radiologic imaging system is the screen–film system, which was first employed soon after the discovery of x-rays. In digital imaging systems, instead of te screen-film, either an imaging plate made of a storage phosphor or flat-panel detectors (FPDs) are used. One of the advantages of digital images is that the images can be changed in many different ways by

use of various image processing techniques. This is a very import advantage, because conventional film images cannot be changed once they have been obtained (1).

Multi-detector CT (MDCT) has been developed to produce hundreds or thousands of axial images with almost isotropic voxel data, thus image interpretation of all individual image slices by radiologists would be prohibitively time consuming. Multiplanar reformatted and 3D images from 3D volume data may be created by use of a surface-rendering or volume-rendering technique. Another approach is to view a large number of these images in a stack (cine) mode for images displayed in the axial, sagittal and/or coronal plane by use of a multi-planar reformatting (MPR) technique (1).

Further improvements will be made, especially in image quality for MRI, ultrasound and molecular imaging which is related to the spatio-temporal distribution of molecular or cellular processes for biochemical, biological, diagnostic or therapeutic applications. The infrastructure of PACS is likely to be improved further in terms of its reliability, speed and capacity. However, CAD is currently still in its infancy, and is likely to be a subject of research for a long time because the successful development of CAD schemes depends on a good understanding of the content of medical images.

Specific contrast agents have been developed for x-ray examinations (mainly CT), sonography and MRI. Most of them are extracellular agents which create different enhancement on basis of different vascularization or on basis of different interstitial network in tissues, but some can be targeted to a particular cell line (e.g. hepatocyte). Iodinated contrast agents are used for CT, gadolinium based contrast agents are used for MRI and microbubbles are used for US (2,3). Microbubbles can be used as carrier for therapeutic drugs which can be released in specific targets under sonographic guidance, decreasing systemic toxicity and increasing therapeutic effect (3).

Contrast media improve diagnostic confidence. Moreover lesion detection, characterization, and assessment of treatment responses are improved. CT -MR angiography and perfusion studies, dynamic imaging generally requires conrtast media. The development of new contrast agents and pathway-specific imaging probes will allow the in vivo elucidation of disease-altered molecular, metabolic, and specific cell cycle functions.

With the current progress in radiology, radiologist and clinicians can obtain more relevant molecular and biochemical information from imaging. For example18[18F]fluoro-2-deoxy-D-glucose (FDG) positron emission tomography (PET) for tumor staging, cardiac perfusion imaging with various nuclear medicine and MR imaging techniques, and MR spectroscopy to characterize brain tumors and prostate cancer are in clinical use since they provide useful biologic, biochemical, or physiologic information that impacts patient management.

The next major area for exploitation is molecular imaging, which will provide more fundamental and important molecular, biologic, and biochemical information. Molecular imaging will enable to characterize and phenotype diseases on the basis of biologic and biochemical, in addition to anatomic, information. Moreover, assessment of the biologic and biochemical alterations such as receptor numbers, pathway regulation, and signal transduction abnormalities, that are present in many disease entities, and analysis of gene, enzyme, and protein abnormalities will be possible (4). Radiologists of the future will have a set of technologies that provides various types of intrinsic information.

B. Molecular Imaging

Molecular imaging can be defined as the in vivo characterization and measurement of biologic processes at the cellular and molecular level. By the use of molecular imaging techniques, the imaging scientist and clinician will visualize physiology and cellular or molecular biological processes in living tissue. Currently available techniques allow visualization and quantitation of potentially relevant physiologic or pathologic and often times molecularly controlled variables such as blood flow, oxygen consumption, glucose metabolism, proliferation, and tissue hypoxia as they take place in living cells and tissues (4,5).

A clinically relevant example is the use of fluorine 18 [18F]fluoro-2-deoxy-D-glucose (FDG) positron emission tomography (PET) and, more recently, that of FDG PET/computed tomography (CT) in cancer diagnosis and management. A molecular or metabolic imaging assessment, , such as PET/CT, provides information that in many instances is of more diagnostic utility than is simple anatomic information.

Molecular Imaging Systems

1. Nuclear Imaging

Nuclear imaging modalities, which include single photon emission computed tomography (SPECT) and positron emission tomography (PET), have the advantages of high sensitivity, unlimited depth penetration, and a broad range of clinically available and clinically tested molecular imaging agents. However, a limitation of PET imaging is its requirement for a cyclotron to generate imaging agents. Additional limitations of nuclear imaging include patient exposure to radiation and its lower resolution (5-10 mm) compared with other molecular imaging systems (6).

Nuclear/ computed tomography (CT) and nuclear/ magnetic resonance (MR) fusion systems (PET-CT, SPECT-CT, PET-MR) integrate the lower resolution molecular information from PET or SPECT with higher-resolution anatomical detail from CT or MR, and are likely to play an increasing role in clinical molecular imaging (6).

2. Ultrasound Imaging

Ultrasound has high spatial resolution (<1 mm) and, can provide excellent anatomical information for coregistration with molecular information. A number of targeted molecular imaging agents have been designed for ultrasound imaging using microbubbles, liposomes, or perfluorocarbon emulsions. An important limitation of the use of ultrasound in molecular imaging is the relatively large size of the imaging agent particles (250 nm), that can restrict tissue penetration and, thus, limit applications to vascular targets (6).

3. Optical Imaging.

An emerging molecular imaging mode is optical imaging, the detection of photons after their interaction with tissue, and in particular, near-infrared fluorescence (NIRF) imaging, which makes use of photons emitted in the near-infrared and farred range. Although penetration of light through tissue is main limitation for all optical imaging methods, attenuation and autofluorescence are minimized in the near-infrared window, permitting deep tissue imaging up to 10 cm^2 (6).

4. Magnetic Resonance Imaging.

The primary advantage of magnetic resonance imaging (MRI) as a molecular imaging system is its ability to provide soft tissue and functional information by using proton density, perfusion,

diffusion, and biochemical contrasts. This feature allows coregistration of molecular information with anatomical information within a single imaging mode.

MRI appears particularly well suited for molecular imaging experiments. It offers high spatial resolution (up to ~50 μm isotropic resolution for high-field MRI), very good soft tissue contrast, and a virtually unlimited depth penetration for preclinical imaging. Moreover, it does not involve ionizing radiation, making it particularly relevant for longitudinal follow-up involving multiple acquisitions. However, it displays limited sensitivity to exogenous contrast agents. MRI presents a sensitivity in the micromolar range. Since very few targets present such a high concentration in living organisms, the development of amplification techniques are mandatory to achieve reliable molecular MRI (mMRI) (6).

Advance imaging research for development, assessment, and validation of new imaging tools, techniques, and assessment methods requires collaboration among imaging scientists and basic biologists, chemists, physicists, and clinicians.

Molecular imaging will be critical for earlier detection and management of cancer in the future. The timeline from initial genetic alterations in the cell to development of clinical cancer can be 40 years or more. With the development of imaging technologies and agents (eg, probes, radiopharmaceutical agents) it may be possible to detect precancerous abnormalities or very small cancers (4,7,8).

Molecular imaging will also play an important role in many genetic, metabolic, neurologic, cardiovascular, pulmonary, and gastrointestinal diseases as well (9-14). For example, molecular MRI may be a valuable tool for noninvasive detection of fibrotic processes and early-stage liver fibrosis (15).

The ability to detect, through imaging, the molecular changes associated with many diseases should vastly improve our ability to detect, diagnose, stage, select appropriate treatments, monitor the effectiveness of a targeted treatment, and determine prognosis in many diseases. Molecular imaging will also be a critical and an integrated part of the drug development process.

Conclusion

Over the last 60 years important progress has been achieved in US, CT, MRI, digital radiography, radyonuclid imaging, image process technique, image storage (PACS), and CAD. Molecular imaging is the next evolving area, and in the future molecular imaging systems will play an important role in early diagnosis and management of many diseases including cancer.

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SONODYNAMIC THERAPY APPLICATIONS

Serçin Özlem ÇALIŞKAN

Abstract

Cancer is one of the most important human health problems in the world. Currently, treatments for cancer comprise four main methods which are clinically applied of surgery, chemotherapy, radiotherapy and immunotherapy. Additionally, each type of treatment has its own limitations creating serious difficulties in cancer treatment. The developing cancer treatment of immunotherapy is an effective treatment method for cancers; however, the cost is excessive. As a result, finding an effective, safe and low-cost treatment has gained great urgency. Sonodynamic therapy (SDT) is a non-invasive, alternative cancer treatment method developed based on the principles of photodynamic therapy (PDT). At the same time, it is a new approach involving a combination of special chemical agents and low intensity ultrasound known to be sonosensitive. The ultrasound used in SDT is observed to form acoustic cavitation as a result of non-thermal effects. The cavitation disintegrates water molecules in the environment and the released free radicals destroy the target cells. At the same time, it is reported that some chemotherapeutic medications may display synergistic effect with low intensity ultrasound.

Introduction

Sonodynamic therapy (SDT) is depend on production of reactive oxygen species (ROS) through simultaneous combination of sonosensitizer, molecular oxygen and low intensity ultrasound. In the absence of ultrasound, the sonosensitizer isn't toxic and the interaction with ultrasound leads to toxic effects in the presence of only molecular oxygen (mediated by ROS) (1).

Sonodynamic therapy was improved as a new, hopeful and non-invasive approach derived from photodynamic therapy (PDT). Some hematoporphyrin (HP) derivatives (HPD) used in PDT by Yumita et al. were found to be activated by ultrasound and cause significant cellular injury (2). Since that time, a few newly-produced HPDs have had potential for use proven as sonosensitizer in combination with ultrasound for tumor treatment called SDT (3-6). The biggest difference between SDT and PDT is the source of energy used to activate the sensitizers. PDT is not effective for treatment of tumors with deep localization, due to the short penetration depth of light (7). Additionally, the most important advantage of SDT compared to PDT is that ultrasound can be tightly focused with penetration a few tens of centimeters into soft tissue (8). As a result, SDT overcomes the main limitation of PDT. The sonodynamic efficiency of SDT is depend on formation of ROS by the simultaneous combination of sonosensitizer, molecular oxygen and low intensity ultrasound (1). SDT is a promising new treatment method providing impressive anticancer effects in both *in vivo* and *in vitro* research (9).

Ultrasound

Ultrasound is a mechanical wave caused by periodic vibrations of particles in a continuous elastic medium at frequencies equal to or higher than 20 kHz. In fluids, it transforms to wavelengths from micrometer to centimeter size at nearly 1000-1600 m/s. As a result, the acoustic area does not directly match the energy levels of molecules, including at molecular level in the biological environment. Hence, this radiation isn't only pick up as safe, but also has the ability to penetrate very well into a tissue without a large reduction in energy. This is a very attractive feature clinically and has led to comprehensive ultrasound assessment for medical purposes (10).

Biological effects of ultrasound

The effect of therapeutic ultrasound on biological tissue is generally divided into thermal and nonthermal effects. Among the thermal effects of ultrasound;

Ultrasound causes an increase in temperature with absorption in an environment. Among the aims of heating tissues are

- Increasing flexibility of collagen fibers in tendons
- Reducing muscle spasm.

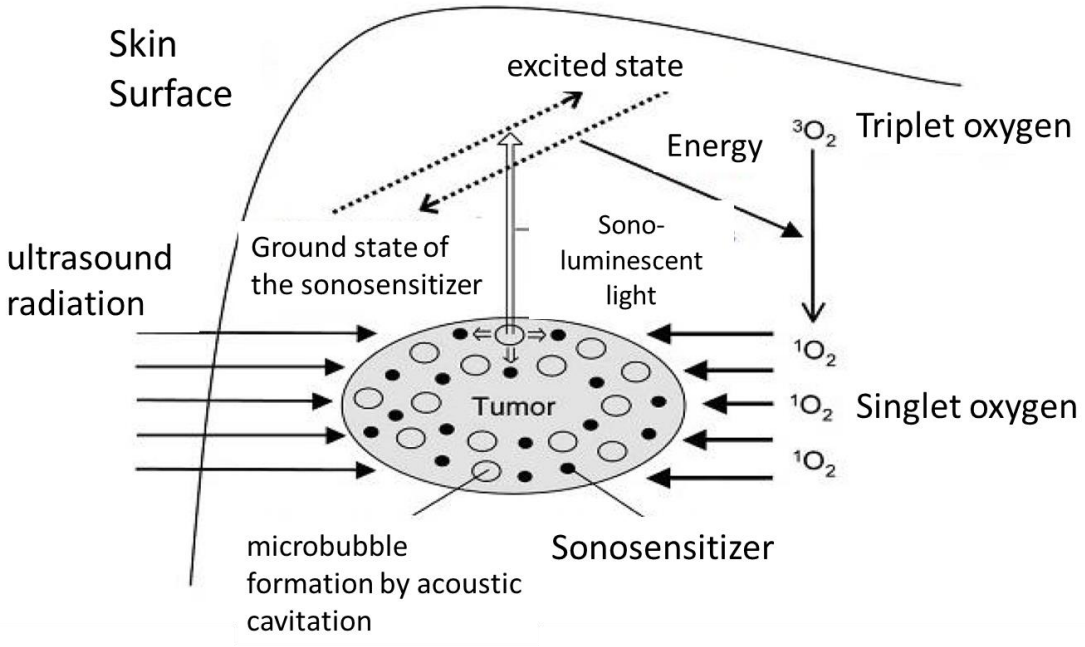
The non-thermal effects of ultrasound include;

- Ultrasonic cavitation
- Free radicals
- Singlet oxygen
- Ultrasound-linked apoptosis
- Situations where combination of several mechanisms are effective.

Cell damage in Sonodynamic Treatment

Research into sonodynamic therapy has focused on the non-thermal effects of ultrasound. The effect of non-thermal ultrasound conducts acoustic energy to the cell surface due to cavitation (11). Cavitation describes formation of microscopic bubbles due to pressure changes occurring during progression of ultrasound through tissue fluids. Cavitation may be classified in two categories as inactive and active. Inactive cavitation is defined as the process of microbubbles being released in tissue fluids, expanding and bursting intensely. In situations where acoustic intensity is suitable to rapidly burst microscopic air bubbles, the energy of the bursting air bubbles activates sonosensitizers and the sonoluminescence event occurs (12). Active cavitation is the situation where micro air bubbles are released with certain shape and size within an acoustic wave and these bubbles burst when the acoustic wave becomes unstable (12,13).

The mechanism of sonosensitizers used during sonodynamic therapy and resulting cell damage is theoretically similar to PDT. The sonosensitizer is stimulated by the acoustic cavitation of ultrasound and provides active oxygen production (14). Sonochemical reactions and the obtained ultrasound radiation stimulate the formation of cavitation around tumor cells. When the sonosensitive agent is stimulated, it passes from basic levels to stimulated levels and the activated sonosensitizer returns to basic levels by releasing energy which creates reactive oxygen species like singlet oxygen and free radicals causing cellular toxicity.



Possible mechanism of cell damage in SDT

Sonosensitizers

Porphyrins developed as first-generation photosensitive agents have a broad area of use in photodynamic therapy. As a result, hematoporphyrin (Hp), Photophyrin®, hematoporphyrin monomethyl ether (HMME), protoporphyrin IX (Pp IX), ATX-70 and many new porphyrin derivatives have been developed as sonosensitizers in the last twenty years. HMME comprises two monomer porphyrins and is both a photo- and sono-sensitive agent associated with hematoporphyrin. HMME localized with high selectivity for tumor tissue has features like rapid removal from tissues and low toxicity. PpIX may accumulate in rapidly proliferating tumor cells and causes death of tumor cells when stimulated by ultrasound. In vitro studies have shown PpIX is an effective sonosensitive agent with great potential for sonodynamic tumor treatment (15). The first in vivo and in vitro studies of the gallium-porphyrin derivative of ATX-70 reported that this agent has sonodynamic antitumor effect in many cancer types (12) Phthalocyanines may chelate with different metals like aluminum and zinc. Location change of sulfonate groups with a ring on phthalocyanines makes these groups water-soluble and increases entry of phthalocyanines into the cell (16). Chloraluminum phthalocyanine disulfonate agent is stated to be a suitable agent for sonodynamic and photodynamic therapies (17). Table 1 gives some sonodynamic therapy implementations.

Table 1. Sonodynamic Therapy Applications

Sonosensitizer	**Biological model**	**Reference**
HMME	C6 glioma cells	18,19
	CNE-2 (*in vitro*)	20
	Osteosarcoma (*in vivo, in vitro*)	21,22
HMME + adriamycin combined		23

PPIX	Hepatoma-22	24
	Sarcoma 180	25
	Sarcoma 180	26
	Sarcoma 180	27
	hepatoma-22 (*in vivo, in vitro*)	28
ATX-70	HL-60	29
aluminum phthalocyanine	G361 in melanoma cells	30
	Colon carcinoma	31,32
	PC-3 prostate cancer	33
Methylene blue	HO-8910 cells	34
acridine yellow	Sarcoma 180	35

Targeting increased treatment efficacy and reduced unwanted side effects, ultrasound energy is assumed to increase the cytotoxicity of chemotherapeutic drugs (Table 2).

Table 2. Chemotherapeutic drugs tested as sonosensitizers

Sonosensitizer	**Biological model**	**Reference**
Adriamycin	Sarcoma 180	36
	Yoshida rat sarcoma	37
	B-16, Melanoma, RIF-1(fibrosarcoma)	38
	MCF-7WT, CHO	39
	CHO	40
	V79 chinese hamster	41
	3AO (human ovarian carcinoma)	42
Cisplatin	Murine renal function	43
5-fluorouracil	Carcinoma	44
	Erhilch carcinoma	45

Conclusion

Studies have shown that SDT has therapeutic efficacy for cancer treatment. Better understanding of the mechanisms underlying ROS formation induced by SDT will make it possible to develop more effective sonosensitizers. This will assist in reasonable control of ultrasound dosimetry and therapeutic response. In the near future, more data required to identify the true anticancer activity of SDT will be obtained and thus, more studies will determine whether SDT is guaranteed to be a new strategy for cancer treatment or not.

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The Republic since its establishment Turkey from the applied health policy in the context of public policy evaluation

Sinan Gürcüoğlu

Abstract

The aim of this study is; public policies that were created for health care by the state in the process extending to the present day is to examine the establishment of the Republic of Turkey in accordance with the historical theme. The best way to understand a public policy correctly is to know the conditions of the period in which that policy was created. Because the reasons for the creation of a policy can also be considered the reason for the existence of that policy. The study, which occurs in the purposes and conditions under which the health policies and policies in Turkey is important in helping to understand the essence. Document analysis method was used in the study. Document analysis method; It is expressed as " the systematic examination of the obtained records or documents as a data source " (Kıral, 2020). The data of the study were obtained by examining all kinds of documents that are accessible in written, visual and electronic media related to the subject. The resulting light of the data in the health field the Republic of Turkey as an important public policies, according to the needs of the day the quality of established policies that vary and a large proportion of these policies are also effectively implemented. Although the economic and socio-cultural inadequacies prevailed in the country in the first years of the foundation of the Republic, it was concluded that health policies were emphasized in the context of combating epidemic and infectious diseases, which affected the majority of the population due to the conditions of the period, and significant progress was made especially in vaccine production and implementation. It has been observed that the foundations of today's health system were laid with the implementation of the " Health Transformation Program" prepared in 2003.

Keywords: Public Policy, Health Policy, Health System.

Introduction

Before the conceptual meaning of health, we can say that it would be more beneficial to know its effect for human and society and what its absence will cost. Especially, the importance of social health becomes more evident during the epidemic periods. Health; Regardless of whether it is at the individual or social level, it can be seen as a prerequisite for the realization of all plans made, every goal desired to be achieved in the short or long term, and every material and spiritual value desired to be achieved. The fact that the Kovid-19 pandemic causes changes in the decisions made in advance, the plans made for the future and the targets to be achieved due to the global effects confirms this. From this point of view, it can be said that only healthy societies consisting of healthy individuals can achieve their goals fully. Because of the importance of health, states in the age we live in aim to protect the health of their citizens and raise healthy generations. For this purpose, they also formulate policies and implement practices. Since the establishment of the Republic of Turkey has set as a priority for health policy and put into practice.

In the context of public policy in health services may be considered necessary to determine a landmark turning point, the beginning of the history of the Republic of Turkey to be said that the organization is the most appropriate time section. The issue of health was at the forefront of the issues that the new state, which was established in a country that has been in a state of war for many years and has just come out of war, should primarily address. Infectious and epidemic diseases constituted the most important health problems of this period and urgent solutions had to be produced. This period, in which the country is in a very difficult economic

situation, appears as a period in which the health system collapses. In this period, when the foundations of many institutions were laid or restructured, important developments were experienced in the health system. Today the basis of the health system, Turkey discarded as equal time with the establishment of the Republic. foundations of today's health care system, have been taken as equivalent to time with the establishment of the Republic of Turkey. It is seen that the newly established state prioritized health policies, therefore, by making important arrangements in the field of health, it implemented public policies that can be considered basic in this period. The fact that the Ministry of Health was one of the first ministries established by the new state confirms this. According to Aydın (2004); The fact that the health organization was established in a country by the state is an indication that the state attaches importance to the health of its people.

It can be said that the most authoritative and determining public organization in the field of health in the country since the foundation of the Republic has been the Ministry of Health. Both public policies on health the formation of both the implementation and monitoring of these policies has been to have a say in the first degree. In 2003, in line with the policies of the 59th Government, it played the most important role in the preparation of the "Health Transformation Program (HTP)" and implemented radical structural changes in the field of health in the country. In the study; First of all, the situation of the health system in the pre-Republican period was examined in general. Then, due to the structural transformation of the health system impact on the country's SDP Turkey's health policy, 1920-2003 Period and is discussed in two separate periods, including the period after 2003. Finally, after the evaluations made, suggestions that may be useful regarding the subject were made.

An Overview of the Pre-Republic Period

Respecting the time required to process of establishment of the Republic of Turkey on the road to knowledge of global and national development, it will be useful for a better understanding of public policy created after the Republic. In the pre-Republic period, the health services provided by the state did not cover the whole of the people, health services were provided only for the palace and army members in this period. Other than these, they had to purchase health services from self-employed surgeons or physicians for a fee. (Çavdar and Karcı, 2014). When considered from this point of view, it can be said that the health system of the Ottoman State has a characteristic that generally continues the practices in the Anatolian Seljuk State. The origins of the hospitals opened under the names of "Darüş-Şifa, Darüs-Sıhha, Bimaristan, Maristan" in some major cities of Anatolia date back to the Anatolian Seljuk State. Apart from these hospitals that were opened by foundations as charity, hospitals were built in cities selected as capital by the Ottoman Empire (Aydın, 2004). Considering the political structure of the states around the world at that time, the lack of social state understanding and the economic development of the countries, it is difficult to evaluate the hospitals opened by the Foundations in Anatolia in the context of a state policy. However, the opening of the "Military Medical School" in 1827 and the "Civil Medical School" in 1864 by the state in the 19th century to train physicians for the army is one of the important developments that can be regarded as public policy in the field of health. Another important development in 1864 was the reorganization of the scope of the "Provincial Regulations" to include all provinces and the implementation of the "Provinces Ordinance". With this regulation, the legal infrastructure of the new organization in the field of health has been prepared. The organization that was formed by the state for the execution of health services and called "Hekimbaşı" was ended in 1849 and a new administrative organization was made in 1850 with the name of "Medical Ministry" (Altıntaş, 2007). In addition, with the effect of the developments in the field of health in Europe , "Haydarpaşa Military Hospital" was established in 1845 and "Zeynep Kamil Hospital" in 1862. In 1886, doctors and veterinarians were sent to Pasteur Institute for training. Returning

to the country in 1887, veterinarians and physicians brought the rabies vaccine with them and produced the first rabies vaccine at Mekteb-i Tıbbiye-i Askeriye-i Şahane. Then, the same year in Turkey "Rabies Treatment Institute" was established (İzgörer, 1998) . "Telkihhane-i Şahane Osmani" was put into service in 1892 in order to produce vaccine to prevent smallpox, which is a contagious disease and significantly affects public health as of the period (Erol, 2003) . It can be said that these developments are important steps in the process of providing health services provided by foundations by the state and spreading them to the general public. Although some policies aiming to spread health services to the general public were put into practice within the scope of reform studies in the 19th century, these policies were not effective in achieving the determined goals, as health services were not counted among the basic duties of the state. (Beylik and others, 2015) . In the Legal Basis of 1876, which was accepted as the first constitution, no statement regarding the field of health was included.

1920-2003 Period

It is seen that the developments in the field of health in the Republican period are generally divided into four or five periods in the literature and analyzed from a historical perspective. However, when looking at the changes in public administration and the ongoing effects of policies in the context of public policies, it can be said that the developments in the field of health can be examined in two periods, from 1920 to 2003 and from 2003 to the present. The establishment of the Republic of Turkey during the period of political, social and economic developments due to be dealt with primarily by the newly established state policies must be produced and has been in the health field. The country's economic weakness due to the war for many years and the long waves of immigration during the war led to an increase in epidemics and infectious diseases. It is known that half of the people living in Anatolia during this period had malaria and a population of nearly three million struggled with trachoma. (Özkaya, 2016) . One of the first acts of the newly established state law No. 3 of 3 in May 1920, " Medic Social Welfare to Temporary of " the establishment has become. During the Ottoman State in the country health services an individual to carry out the facing health has not been established. Health care is a period, " the Foreign Ministry of " term also " Interior Ministry for" on-site was carried out (Tekir, 2019). The establishment of an independent ministry in the field of health has been one of the first in the country in terms of state organization. During the period of Adnan Adıvar, who was the first Minister of Health, the issue of combating epidemic and infectious diseases was one of the issues that were prioritized by the state and produced public policy. Smallpox vaccine was first brought from Italy, the tuberculosis sanatorium was opened in Burgaz Island, the bacteriology department for rabies treatment and the vaccine house were established in this period. In the 5-year period from the establishment of the Ministry of Health to 1925, 150 dispensaries were built across the country and significant progress was made in the fight against tuberculosis. Plans have been prepared to combat diseases such as syphilis, malaria, rabies, and trachoma, the shortage of doctors has been reduced, and necessary medical devices and medicines have been provided (Gül, 1988). While policies aimed at solving health problems in the country were produced, policies in the field of health education were also produced due to the lack of sufficient health personnel. In 1928, the "Sanitation Institute" was established, in 1930 the "Law on the Practice of Medicine and Medical Arts" and the "Public Health Law" were enacted. (Karabulut, 2007). In Article 1 of the Law; It has been stated that health services are the main duty of the state, with the statement "Improving the sanitary conditions of the country and fighting against all diseases or other harmful agents that harm the health of the nation, and ensuring the well-being of the future generation and giving the public medical and social assistance" (Umumi Hıfzıssıhha Kanunu, 1930). The law also includes measures to be taken for the protection of health and especially to combat contagious and epidemic diseases, and the "Ministry of Sıhhiye Muavenet-i İçtimaiye" has been authorized and

responsible for the implementation and supervision of these issues. With the law, a central organization in the field of health has been established in provinces, districts and districts, which are still valid today. “Health and Social Assistance Directorates”, which are the first forms of today's health directorates in provinces, and Government Doctorships in districts and districts ”were established. Two "Medical Officer Schools" were opened in Istanbul and Sivas to train health officers. It was decided to organize national medical congresses, and the main themes of these congresses, the first of which was held in 1925 and held 11 times until 1950, were infectious and epidemic diseases. (Aksu, 2006) .

Vaccine production, which started in the Ottoman period, continued after the declaration of the republic. Vaccine production, which started in the Ottoman period, continued after the declaration of the republic. With the establishment of the Hıfzısıhha Institute in 1928 and the enactment of the "General Health Law" in 1930, vaccine production and application was centralized and routine vaccination services began to be provided in 1930. In the 1940's, serum production and vaccine production and application were institutionalized and mass production of "rabies, typhoid, tetanus, typhus, cholera, diphtheria, pertussis, BCG, influenza and typhoid-diphtheria-tetanus mixed" vaccines was realized. (Aşı Portalı, 2020). The cholera outbreak originated in China in 1940, the cholera vaccine produced in Turkey as a result of effective policies and practices in vaccine production is thus ensured exported to China. Likewise, the participation of Turkey, but that of the many countries and lasted until the Second World War in 1945, Turkey in 1939, many countries have met the needs of typhus vaccine. (İzgörer, 1998). Liberal policies began to be effective throughout the world since 1945. Turkey also experienced significant improvements in the health field with the effects of liberal policies during this period, including a plan for each semester of the period between the years 1946-1948 and 1954-1955 Minister of Health Dr. Two "plans" have been explained by Behçet Uz. The first of these plans is the "First Ten-Year National Health Plan" announced in 1946, and the other is the "Studies on the National Health Program and the Health Bank" plan, announced in 1954. (Uzi, 1954) . Although the plans are not enacted, they have been the source of structural changes and transformations in the health system. Both plans have the characteristic of being a roadmap for public policies produced in the field of health both in the period they were announced and in the following periods. The plans had a direct impact on the health policies created especially from the 1960s to the 1980s. The Law on Socialization of Health Services was put into effect in 1961 in order to ensure that health services are provided and socialized in accordance with social justice (Sağlık Hizmetlerinin Sosyalleştirilmesi Hakkında Kanun, 1961).

The upheavals across the world have demonstrated the effects of public health administration in Turkey as well and prepared after the coup 1980 1982 Constitution, the state removed from the state's constitutional duties, planning and supervision has been assigned to the field of health (Türkiye Cumhuriyeti Anayasası, 1982) . With these developments, which can be seen as a reflection of the changes in the understanding of public administration, private sector investments in the field of health have gained momentum. The "Health Services Basic Law", which aims to make important changes in the organizational structures of health institutions, was enacted in 1987, but the law was not fully implemented. (Soyer, 2000). When evaluating public policies based on privatization policy in this period is generally set forth in the health field the heavy said that the bass. In the "Sixth Development Plan" covering the years 1990-1994, in the "National Health Congresses" held in 1992 and 1993, road maps for the implementation of these policies were drawn. “Green Card” and “Family Medicine” practices implemented in this period are among the developments that can be considered important. Also belonging to the state of health care institutions gradually privatizing policies also again been created in this period.

2003 and After

Since 2003, Turkey in the field of health practices that can be considered and several policy reforms began to be implemented still valid today was created during this period. Founded in 2003, the 59th. one of the first actions of the government was to determine the problems experienced in the field of health and to determine the actions to be taken to eliminate these problems. The state of the health system in the country in the government program, which was read in the parliament by Recep Tayyip ERDOĞAN, the Minister of the period, "The current health system has fallen behind modern developments in all aspects ; costs have increased significantly due to system leaks, health services have become unreachable, and there is no standard unity. In order to raise a healthy generation, it has become inevitable to make health services accessible to all citizens. " as if a was in (Türkiye Büyük Millet Meclisi, 1999).

The basic text that serves as a source for the health policies of this period is the "Health Transformation Program (HTP)" published by the Ministry of Health in 2003. Health policies of the 2000s were created under the guidance of the HTP . The program was prepared with a human-centered approach and radical structural changes in the field of health were planned. In addition, the program included the continuity of policies, efficiency and efficiency in services, ensuring the participation of different circles, devolution of power to the local, separation of powers in health (planning, offering and financing) (Memişoğlu, 2018). The main purpose of the program is; " Health services for the effective, efficient and equitable way to be organized in accordance with these services, healthcare provision and funding submission " respectively. The 8 topics determined within the scope of SDP are as follows;

"1. Planning and Supervisory Ministry of Health,
2. General Health Insurance that gathers everyone under one roof,
3. Widespread, easy to access and friendly healthcare system,
    a) Strengthened primary health care and family medicine,
    b) Effective, gradual referral chain,
    c) Healthcare enterprises with administrative and financial autonomy,
4. Health workforce equipped with knowledge and skills and working with high motivation,
5. Educational and scientific institutions to support the system,
6. Quality and accreditation for qualified and effective health services,
7. Institutional structuring in rational medicine and supplies management,
8. Access to effective information in the decision process: Health Information System " (Sağlık Bakanlığı, 2012).

In line with the SDP, the organizational structure of the Ministry of Health has been reconstructed. The Ministry has been determined as the most authoritative institution in the formulation of health policies, in determining the basic principles and rules in the field of health and in their control. New institutions have been established for the execution of these duties and powers assigned to the Ministry of Health and these institutions have been attached to the ministry. The names and establishment purposes of the new institutions affiliated to the Ministry are as follows;

1. "Public Health Agency of Turkey"; To carry out preventive and primary health services,
2. "Turkey Public Hospitals Authority"; Establishing, operating and, when necessary, merging or closing health institutions,
3. "Turkey Pharmaceuticals and Medical Devices Agency"; Making regulations and inspections regarding drugs and medical products (Seçtim, 2019).

HTP has also been a source of many innovations in the field of health, as well as in the field of social security. Within the scope of the implementation of the HTP, the Social Security Council (SGK) was established and health financing was gathered under an additional roof in order to provide services from a single source. Family medicine has been switched to the system, primary health service in for the whole society in the run services to provide planning

and understanding of the individual as a reference year the services provided to patients apan had been passed to the system run. Public health unions have been established. Within the scope of financial innovations, the payment of shares from the revolving fund and performance-based wage payment practices were implemented, and wage policies were re-created to ensure competition among healthcare workers (Soysal and Yağar, 2015).

Public policies have been established and implemented by the governments established after 2003 in order to implement the plans planned with the HTP. This is the beginning of the implementation of all citizens can be treated free of charge in all public and private hospitals come. KDV rates in drug prices, drug prices decreased so that a general discounts provided, and villages were facilitated access of citizens living in villages with drug drug administration. The health record application was removed and hospitals were enabled to switch to the appointment system. With the practice of family medicine, it has become possible to follow up patients individually on a regular basis. A “Central Physician Appointment System (MHRS)” was created, thus enabling citizens to make appointments over the phone or online, and to select a doctor and a hospital. In order to enable patients to share their complaints and problems quickly and directly, a corporate phone line called “ALO 184” is also provided to provide consultancy services to patients. In addition, a patient rights unit has been established in all state hospitals.

One of the important regulations made within the scope of the implementation of HTP has been the "Public Private Partnership (PPP)" model. This model generally; Health investments to be made by the public are made by the private sector and leased to the state for a certain period of time. With the implementation of the model, the investment expenditures of the public sector in the field of health are undertaken to a certain extent by the private sector, thus reducing the burden of the public sector. The purpose of the application; It is aimed to ensure efficiency in health services, to increase efficiency, to diversify treatment services and to spread them across the country at an affordable cost. "Integrated health campuses" and "city hospitals" are among the practices implemented based on this model. Until 2020, 11 city hospitals have been opened throughout the country, 2 hospitals have come to the stage of opening and 5 city hospitals are planned to be opened in 2021. At the same time, many contracts have been signed going forward. (Sağlık Bakanlığı, 2020).

CONCLUSION AND RECOMMENDATIONS

Turkey is seen that practices in the area of health based on the anniversary of the Republic of origin when taken in the context of overall public policy. Due to the peculiar characteristics of the Ottoman state system, pre-Republic health practices cannot be evaluated in the context of public policy. If it is reduced to private and looking at today's results, it can be said that today's health system is based on the Health Transformation Program (HTP), which was implemented gradually in 2003 and after.

The state of the country in the first years of the Republic; We can define it as a country that has been in a state of war for many years and finally got out of the war. After these long wars in the country, most of the surviving soldiers returned to their hometown as either disabled or sick. Epidemic and infectious diseases have increased in this period when the majority of the population has been relocated and the provision of medicine and treatment services has become very difficult in the country, which is in an economically difficult situation. In this period, many infectious diseases such as malaria, rabies, trachoma, syphilis and smallpox are being struggled around the world, for which there is no vaccine, medicine or any treatment method yet. Turkey in this period that the creation of a time in the field of health necessary public policy can be

considered short, in this direction, many laid the foundation of new institutions, the preparation of the necessary legislation, it appears that epidemic and infectious diseases by effectively be combated.

Developments in the field of health throughout the world have also affected Turkey's health policy. In particular, the weight of the liberal economic policies that effect after the 1980s showed the impact on Turkey's health system. this is the period of structural change and transformation in public management, downsizing and reducing the weight of the public in Turkey that has been a period of more flexible and efficient structure to ensure that it has the quest. In this context, privatizations have been on the agenda of the country for many years and private sector investments have been encouraged in the field of health. The number of private health institutions in the country has increased rapidly, and the public-private partnership model has allowed the private sector to take place more in the field of health.

The 58th Government, which came to power in 2002, remained in office for a short time, and then the 59th Government, which took over in 2003, quickly established public policies in the field of health, which it regards as one of the areas where urgent solutions are needed. It has prepared the "Health Transformation Program", which is a road map for the works to be done with priority. The program has been prepared with a wide range of stakeholder participation and appears to be the most comprehensive and inclusive program ever made. The program, which is the source of the current health system applications, has been implemented successfully and to a large extent. It can be said that the existence of political stability in the country and the continuation of the governments that prepared and implemented the program contributed greatly to the success in the implementation of the program.

In the study; It has been observed that the governments established since the foundation of the Republic have dealt with the field of health as a priority, and have significantly included the field of health in their programs and plans they have prepared. In the country, radical and structural transformations have been experienced in the field of health after 2003. It has been observed that technological developments have been utilized in the provision and distribution of medicines, as well as in the fair distribution and provision of healthcare services across the country, enabling the public to access health services more easily. The study reveals the historical background of Turkey's health care system is important for researchers interested in the topic. Turkey's epidemic and infectious diseases in line with public policies that create struggle, more in-depth and detailed manner by addressing public policy analysis methods of process analysis and investigations suggested. In this way, it is thought that it can contribute to the process of creating a public policy regarding the measures to be taken by the state in combating the covid-19 epidemic, which affects the whole world today.

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Transfusion Center Management in Covid-19 Pandemic

Serdar GÜNGÖR

In addition to the historical epidemic regarded to arise withinside the Morea War in 430 BC, epidemics have affected many civilizations at some stage in human records. Justinian Plague (541-750 AD), Black Death (1347-1351), Cholera (1817- 1823), Smallpox (15th - 17th Centuries), Spanish Flu or H1N1 (1918-1919), Hong Kong Flu or H3N2 (1968-1970), HIV / AIDS (1981 - present), SARS (2002-2003), Avian influenza or (H5N1) 2005, Swine Flu or H1N1 (2009-2010), Ebola (2014-2016) outbreaks were among the most important.

Since unknown pneumonia affected person became discovered December 2019 in Wuhan, China, a novel new coronavirus (CoV), which became briefly named 2019 novel coronavirus (2019-nCoV) with the aid of using the World Health Organization (WHO) on January 7, 2020 (1). The virus became sooner or later renamed Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), and the ailment it reasons became named Coronavirus Disease 2019 (COVID-19). The World Health Organization declared COVID-19 in China as a Public Health Emergency of International Concern. Two different coronavirus infections, SARS in 2002-2003 and Middle East Respiratory Syndrome (MERS) in 2012, each caused excessive breathing syndrome in humans. All three of those rising infectious sicknesses main to a worldwide unfold are because of β-coronaviruses (2, 3). In China, previous outbreaks of rising infections have had an unfavourable effect on the blood supply. However, attention should additionally receive to the protection of the transfusion recipient even supposing the rising contamination is a breathingailment. Previous research indicated that viral RNA will be detected from plasma or serum of sufferers inflamed with SARS-CoV, MERS-CoV, or SARS-CoV-2 for the duration of exceptional durations after the onset of signs (1, 4). With extra and extra asymptomatic infections being discovered amongst COVID-19 cases, blood protection is worth of attention.

As the most important regarded RNA viruses, CoVs are in addition divided into 4 genera: α-CoVs, β-CoVs, γ-CoVs, and δ-CoVs, among which α- and β-CoVs are capable of infect mammals, while the different genera can infect birds and can also infect mammals (5). SARS-CoV-2 became these days remoted from human airway epithelial cells, characterised with the aid of usingnext-technology sequencing in January 2020, and diagnosed to be a brand new member of β-CoVs (6). The viral content material of plasma became low. Researchers should simplest discover SARSCoV RNA use of a nested PCR assay installed in-house, and the viral load became 190 copies/mL finished after ultracentrifugation of 2 mL of plasma. Based in this examine and different facts, WHO and the US Food and Drug Administration (FDA) drafted hints on blood protection and mentioned the atheoretical hazard of transmission of the SARS virus via transfusion of blood merchandise (7, 8). Inactivation of Coronavirus in Blood Products Coronaviruses is enveloped, positive-sense, single-stranded RNA viruses. Usually, coronaviruses are liable to acid-pH, basic-pH, and warmth however appear to be extra strong at 4°C (9, 10).

In the Current Situation Statement published by the WHO "Blood Regulators Surveillance Network" on January 28, 2020, when vaccine and / or effective anti-viral drugs are not available, as previously applied in the MERS epidemic, immune plasma, serum or immunoglobulin concentrates are not available for SARS-CoV-2 It is stated that it can also be used for the virus. In this regard, it was emphasized that the competent authorities should establish the necessary arrangements for the collection of immune plasma or serum. (11, 12)

Donor and staff safety; in an effort to maintain the inventory, the safety of donors and staff cannot be put at risk. Voluntary donors residing at a walk-away distance from the blood bank too might be apprehensive to turn up for donation. The blood collection sites and stations need to undergo thorough disinfection and disposal of medical waste needs be handled more meticulously. Psychological impact of COVID-19 as a disease and the effects due to the nationwide lockdown on donors, acute or long-term, would also require attention. Due to mobility constraints, daily commute of employees is affected. Also, employee absenteeism can be expected due to illness of self/ family or panic especially among those handling infected patient's sample. As health-care workers who are the front-line warriors are at not only at a higher-risk of contracting the disease but may also experience adverse psychological issues like burnout, anxiety, depression, and PTSD (Posttraumatic stress disorder) amongst many. These issues need to be handled with patience and care (13). Initiatives like virtual extracurricular courses on self-care and offering online psychiatric support will help in the emotional well-being of healthcare workers (14). All infectious diseases can be passed on to human or animal in one or more ways. For this reason, as in other endemics, it is effective disinfection to stay away from contact and contamination routes, to protect the contaminated areas of the body in the fight against Covid-19. It is also important that preferences such as mood, belief, nutrition, sleep regime, and lifestyle that affect immunity are compatible with universal general truths.

Covidien-19 also seen in this frightening situation, we can do the transfusion center: Turkey blood centers and transfusion association of (KMTD) made by Covid-19 (2019-nCov) donations of pandemic period, blood components, and recommendations for transfusion in our hospital were taken into consideration by the Central transfusion and tried to practice (15-17).

1. Recommendations for donor selection and donation process:

• Donors were not summoned to the blood service unit collectively, an appointment was made.

• The conditions of isolation were provided in the waiting, donation, and resting rooms (distance, hand sanitizer, mask, etc.).

• As a precaution, donors with a history of symptomatic flu in the last 28 days did not receive blood.

• During the interrogation, we paid attention to the flu symptoms.

• We questioned if there is a history of contact with someone diagnosed with COVID-19.

• Those with fever, runny nose, sneezing, cough, muscle pain, headache, sore throat, and shortness of breath were not accepted as donors.

• It was said that the donor should inform your blood service unit in case of fever, cough, cold, loss of taste and smell, flu-like symptoms within 48 hours after donation.

2. Recommendations for clinical use of blood:

• Informing our hospital's clinics about the blood donation measures taken was done as much as possible from both the in-hospital computer system and social media groups.

• Although the transmission of SARS-CoV-2 by transfusion is a theoretical risk, the principle of "no evidence of risk is not proof of no risk." Based on this, clinicians who requested blood and blood products were informed.

• Exclusion of risky donors and quarantine application of blood components are the leading measures to reduce the risk of transmission of SARS-CoV-2. The clinics were informed about avoiding unnecessary transfusion in order to avoid any difficulties in obtaining blood products.

• Elective surgeries were postponed by the decision of our Ministry.

• The Transfusion Committee convened, formed a hospital pandemic transfusion policy, and was informed about the management of the decisions taken.

3. Recommendations for blood service staff and visitors:

• Necessary information was given to the responsible managers for family members diagnosed with COVID-19 and those who had contact with their relatives.

• Certainly, no visitors from outside were accepted to the blood service unit.

• Adhere to biosecurity measures and training and warnings were given for the use of personal protective equipment, moisture barrier gowns, protective glasses and masks were provided from the hospital administration.

• Glasses, gloves and N95 / FFP2 mask were used by laboratory staff working with patient samples and a moisture barrier overall was worn. Since the centrifugation process may cause virus spread by aerosolization, it was strongly warned not to open the centrifuge lid immediately at the end of the procedure.

• Personnel who came to take blood and blood products were not allowed in, a distance barrier was provided by placing a table in front of the door.

Serological tests will be insufficient in screening symptomatic or presymptomatic donors, as no detectable antibodies will be formed during the acute infection phase. If donor samples are to be screened for SARS-CoV-2, this could possibly be nucleic acid tests (NAT) or polymerase chain reaction (PCR), which are known to be costly. If the transmission of SARS-CoV-2 by transfusion is shown in the future, the use of commercial pathogen reducing technologies, which have been shown to be effective for Coronavirus, in the preparation of blood components may be an option.

As a result, there is no treatment method, vaccine, hyperimmunglobulin yet available for COVID-19. Under these conditions, the most effective supportive treatment method that can be done while new treatment agents are rapidly researched and developed in the world and in our country is the application of convalescent (immune) plasma. Studies have shown that this passive antibody treatment, which has been shown to be effective in previous epidemic periods in history, gives positive results in the COVID-19 pandemic. It has been started a very short time ago in our country. Until an effective and safe treatment method is found, other current treatment methods (antiviral, anticoagulant, etc.) seem appropriate to be applied for support purposes.

Plasmapheresis methods can be used to remove cytokines and harmful metabolic residues in patients with multiple organ dysfunction due to SARS-CoV-2 infection. An artificial liver support system is one of them. There are publications stating that the plasma of people who have become immunized after SARS-CoV-2 infection, that is, antibodies in their plasma, can be used for passive immunization in treatment (18, 19). More than 130 units of products have been provided to our intensive care patients since the beginning of the pandemic, especially regarding immune plasma.

Blood is a constant need that, unfortunately, has no alternative. Blood products need to be renewed constantly due to their narrow shelf life. Although this need is not expected to increase due to the pandemic, especially in terms of the use of plasma and platelets, there has been a decrease in blood donation due to the pandemic and difficulties have been experienced in meeting the need for blood components. During the pandemic, in order to ensure the balance of blood product needs and stock, "clinical use of blood" should be properly implemented and brainstormed with clinicians while the donation process continues.

While it is not practical to keep the nation under Stay At Home Orders for extended periods of time, enforcing social distancing measures in the long-term is a realistic option that has evidence of improvement of the national blood supply.

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INJURY CONTROL IN CHILDHOOD

Şule YILDIRIM

Injuries are the most common cause of death during childhood and adolescence beyond the 1st few mo of life and represent 1 of the most important causes of preventable pediatric morbidity and mortality (Figs. 1 and 2). The identification of risk factors for injuries has led to the development of successful programs for prevention and control. Strategies for injury prevention and control should be pursued by the pediatrician in the office, emergency department, hospital, and community setting.

The term accident prevention has been replaced by injury control. The word accident implies an event occurring by chance, without pattern or predictability. *Accident* connotes a random event that cannot be prevented. The use of the term injury promotes an awareness of a medical condition with defined risk and protective factors that can be used to define prevention strategies.

There are three steps in preventon of injury control and the term «injury control» describes all of these steps

These steps are classified as primary, secondary and tertiary. While it is the basic to avert the event or injury in the first place, the secondary and tertiary preventions include appropriate medical services, regionalized trauma care and rehabilitation services after injury

İntentional injuries such as self-inflicted injuries which are important in adolescents and young adults are also included in this expanded definition.

In the USA, injuries cause 42% of deaths among 1-4 yr old children and 65% of deaths among the rest of childhood and adolescence up to the age of 19 yr

Motor vehicle injuries are the leading causes of injury deaths at all ages during childhood and adolescence.

Drowning is the 2nd cause of unintentional trauma deaths, with peaks in the preschool and later teenage years. In young children, bathtub and swimming pool drowning predominate, whereas in older children and adolescents, drowning occurs predominantly in natural bodies of water while the victim is swimming or boating.

Fire and burn deaths account for 4% of all unintentional trauma deaths. Most of these are due to house fires; deaths are caused by smoke inhalation and asphyxiation rather than severe burns. Children and the elderly are at greatest risk for these deaths because of difficulty in escaping from burning buildings.

Suffocation accounts for approximately 73% of all unintentional deaths in children younger than 1 yr of age. The majority of these deaths result from choking on food items, such as hot dogs, candy, grapes, and nuts. Nonfood items that can cause choking include undersized infant pacifiers, small balls, and latex balloons.

Homicide is the 3rd leading cause of injury death in children 1-4 yr of age and the 2nd leading cause of injury death in adolescents (15-19 yr old). Homicide in the pediatric age group falls into 2 patterns: infantile and adolescent. Infantile homicide involves children younger than the age of 5 yr and represents child abuse. The perpetrator is usually a caretaker; death is generally the result of blunt trauma to the head and/or abdomen. The adolescent pattern of homicide involves peers and acquaintances and is due to firearms in >80% of cases. The majority of these

deaths involve handguns. Children between these 2 age groups experience homicides of both types.

Suicide is rare in children younger than age 10 yr; only 1% of all suicides occur in children younger than age 15 yr. The suicide rate increases markedly after the age of 10 yr, with the result that suicide is now the 3rd leading cause of death for 15-19 yr olds. Approximately one half of teenage suicides involve firearms.

Nonfatal Injuries

Falls are the leading cause of both emergency department visits and hospitalizations. Bicycle-related trauma is the most common type of sports and recreational injury. Nonfatal injuries, such as anoxic encephalopathy from near-drowning, scarring and disfigurement from burns, and persistent neurologic deficits from head injury, may be associated with severe morbidity, leading to substantial changes in the quality of life for victims and their families.

Who is Most Vulnerable?

Some children are at greater risk than others for an injury. Injury-related death and disability are more likely to occur among males, children of lower socioeconomic status, those living in specific geographic regions, and in certain racial/ethnic groups.

Gender

• In every age group across all races and for every cause of unintentional injury, death rates are higher for males.

• Male death rates are almost twice that of females.

• Males aged 15–19 years have the highest rates of ED visits, hospitalizations, and deaths.

Race/Ethnicity

Age

• Children less than 1 year of age who die from an injury are predominantly victims of unintended suffocation or accidental strangulation.

• Drowning is the main cause of injury deaths among children aged 1–4 years.

• Most deaths of children aged 5–19 years are due to traffic injuries, as occupants, pedestrians, bicyclists, or motorcyclists.

Socioeconomic Status

• Children whose families have low socioeconomic status or who live in impoverished conditions and are poor have disproportionately higher rates of injury.

• A broad range of economic and social factors are associated with greater child injury including:

» Economics: lower household income.

» Social factors: lower maternal age, increased number of persons in household, increased number of children in household under 16 years, lower maternal education, single-parents.

» Community: multi-family dwelling, over-crowding, and low income neighborhoods.

Geography

INJURIES ARE NOT ACCIDENTS

Injuries are often understandable, predictable, and preventable

Specific injuries share similar characteristics of person, place , and time

By understanding injuries, interventions can be developed and implemented to prevent or limit the extent of a given injury

William Haddon and the Phase Factor Matrix

First conceptual framework for studying injuries causes and prevention, developed by William Haddon

By studying a specific injury with this matrix in mind, one can identify **modifiable** risk factors and identify points of intervention in the causal sequence

Much like an infectious disease:

Host=person experiencing injury

Vector=e.g. a bicycle or car

Environment=physical and socioeconomic condition surrounding event

Three Phases during which each factor must be evaluated:

pre-event phase

event phase

post-event phase

NOTE - Injury deaths follow a trimodal distribution: 50% immediate deaths, 30% early deaths (within the first 3 hours), 20% late deaths. 80% occur with the first three hours. Primary prevention in the key!

Child injuries are preventable

Implementing interventions could save more than 1000 children's lives a day.

Report describes 24 proven interventions.

Many high-income countries have been able to reduce their child injury deaths by up to 50% over the past three decades by implementing multisectoral, multi-pronged approaches to child injury prevention.

Children are at greater risk

Children are not just little adults.

They live in a world built for adults.

Strong association between injuries and

A child's age

Developmental stage

How he/she interacts with the world

Activities undertaken

Child injury prevention is cost effective

In a survey conducted in the late 1990s on the costs of childhood unintentional injuries and the cost-effectiveness of interventions to prevent them showed that approximately 15% of medical spending resulted from an injury. The same study found that seven child injury safety measures – child safety seats, bicycle helmets, zero tolerance of alcohol for young drivers, provisional licensing, smoke detectors, childproof cigarette lighters and poison control centres – had similar cost-effectiveness ratios to other well accepted strategies to prevent childhood illness. The implementation of these strategies, though, is not yet widespread. As can be seen from Table many cost-effective strategies for unintentional injury can save not only lives but costs to society as well.

Strategies for Prevention

Intervention or countermeasures are classified based on requirements for behavior change

Active - rely on actions taken by an individual (e.g. storing meds in high/locked cabinets)

Passive - do not rely on the efforts of an individual to be successful (e.g. packaging meds in nonlethal amounts/child safety caps)

Methods of Prevention - Three "Es"

Engineering

Environmental change

Education

FACTS AND ACTS

Road traffic injuries: Facts

720 children die from road traffic crashes every day.

Globally, road traffic injuries are the leading cause of death among 10-19 year olds.

In low-income and middle-income countries most traffic deaths are among pedestrians, passengers in vehicles or on two-wheelers.

In high-income countries most traffic deaths are novice drivers.

The most common non-fatal injuries sustained by children are head injuries and fractured limbs.

Road traffic injuries are a leading cause of disability for children.

Road traffic injuries: Acts

Minimum drinking-age laws.

Lower BAC (**blood alcohol** concentration) limits for novice drivers and zero tolerance.

Graduated driver licensing systems.

Helmets.

Seat-belts, child-restraints.

Speed reduction.

Separating road users.

Daytime running lights.

Drowning: Facts

480 children die from drowning every day.

Each year 2-3 million children and teenagers get into trouble in water and come close to drowning.

Globally children under the age of 5 years are at greatest risk of drowning – infants can drown in a few centimetres of water.

Over 98% of child deaths from drowning occur in low-income or middle-income countries, usually in open bodies of water like lakes, streams, etc.

In high-income countries, most drowning events happen in swimming pools.

Drowning: Acts

Removing (or covering) water hazards.

Requiring isolation fencing (four-sided) around swimming pools.

Wearing personal flotation devices.

Ensuring immediate resuscitation.

Burns: Facts

260 children die from a fire-related burn every day.

The death rate from burns is 11 times higher in low-income and middle-income countries than in high-income countries.

Infants are at highest risk of death from burns.

Burns is the only type of injury which is more common among girls than boys (particularly in adolescence).

Smoke inhalation from fire-burns can be deadly.

Nearly 75% of non-fatal burns are from hot liquids, hot tap water or steam.

Many children are disfigured for life from burns.

Burns: Acts

Setting (and enforcing) laws on smoke alarms.

Developing and implementing a standard for child-resistant lighters.

Setting (and enforcing) laws on hot tap water temperature, and educating the public.

Treating patients at a dedicated burns centre.

Falls: Facts

130 children die from a fall every day.

60% of these fatal falls are from a height.

In some countries, nearly half of the children taken to emergency clinics are from falling.

Non-fatal falls result in significant Disability Adjusted Life Years lost.

Falls most commonly occur from:

Prams, baby walkers, changing tables,

Cots, beds, bunk beds;

Rooftops, windows, stairs;

Playground equipment;

Trees; and

Sports.

Falls: Acts

Redesigning nursery furniture and other products.

Establishing playground standards for the depth of appropriate surface material, height of equipment and maintenance.

Legislating for window guards.

Implementing multifaceted community programmes such as ‘Children can't fly’.

Poisoning: Facts

125 children die from poisoning every day.

The rate of poisoning is highest for children under 1 year, but peaks again at 15 years and older as adolescents begin experimenting with substances.

Fatal poisoning rates are 4 times higher in LMICs than HICs.

The most common poisoning agents in LMICs are paraffin, household products and pharmaceuticals.

In HICs the most common poisons are over-the-counter medications, household products, and prescription drugs.

Poisoning: Acts

Removing the toxic agent.

Legislating for (and enforcing) child-resistant packaging of medicines and poisons.

Packaging drugs in non-lethal quantities.

Establishing poison control centres.

If we look at this picture we can see a lot of modifiable risk factors to prevent injury.

1-toll on the stairs

2-the door to stairs is open

3- Wall plaster is damaged

4-electrical outlet is open

5- hot iron is at accessible point

6- cleaning products are accesible

7- oven is open

8- pot handle is everted

9-a footstool next to the oven

10- scissors is accessible

Simple design of household features and the removal of the hazards from the home can help prevent the occurence of serious and often fatalinjuries to children at home

You Can Make a Difference

Injuries are not accidents

Gather your "stories" to help give advice

Patients do listen

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Tuba DAL

Intestinal microbiota and gut-lung axis

The human intestinal microbiota consists of 10^{14} resident microorganisms, including bacteria, archaea, viruses and fungi. Actinobacteria, Firmicutes, Proteobacteria and Bacteroidetes, are dominant in healthy individuals. In the colon, families of Bacteroidaceae, Prevotellaceae, Rikenellaceae, Lachnospiraceae and Ruminococcaceae are quite dense. Microbiota members benefit from the host in terms of habitat and food. On the one hand, they mediate the physiological functions of the host and provide protective immunity against pathogens. Gut microbiota changes, called intestinal dysbiosis, have been reported to have a potential role in the emergence of inflammatory bowel disease, type 2 diabetes mellitus, cardiovascular diseases, and neurological and psychiatric diseases (1). In recent years, it has been claimed that the lung also has a microbiota including Bacteroidetes, Firmicutes and Proteobacteria, dominantly (2,3). A vital two-way communication between the gut and the lungs called the "intestine-lung axis" is mentioned (4). Endotoxins, microbial metabolites in the intestine can pass through the blood to the lungs and affect the lung; inflammation in the lung can also affect the gut microbiota (5). The pneumonia and acute respiratory distress syndrome (ARDS) are significant problems especially in elderly and immunocompromised Covid-19 patients (6). Manu studies have reported that gut microbiota plays a key role in the pathogenesis of sepsis and ARDS (7). In the elderly people, the diversity of intestinal microbiota is low and beneficial microorganisms such as Bifidobacterium have decreased (8). The evidences of many elderly and immunocompromised patients with Covid-19 supports the hypothesis that there is a communication between lung and gut microbiota (1).

Gut microbiota and immunity

It is assumed that the gut microbiota significantly mediates the development and function of innate and acquired immune systems. Intestinal commensals secrete antimicrobial peptides, compete for nutrients and habitat and thus aid homeostasis. The signals caused by the gut microbiota prepare immune cells for pro-inflammatory and anti-inflammatory responses; affects predisposition to various diseases. The balance of Treg (inflammatory regulatory T cells) and Th17 is important in the regulation of the immune system, and the microbiota is important in maintaining this balance. There is a close, complex relationship between Th17 and Treg cells. These cells come from a common origin (naive T cells) and most of the chemotactic receptors on their surface are similar. However, while Th17 cells mediate the inflammatory response; Tregs mediate immune tolerance. Under normal conditions, a dynamic balance exists between Th17 and Treg cells. This balance causes a stable immune state to be maintained in the body. It has been reported that the imbalance in the Treg / Th17 ratio is closely related to the immune system disorders, tumors and infectious diseases. A healthy intestine microbiome prevents excessive, harmful immune responses for the lungs and other systems, and in provides an optimal immune response in infections such as coronavirus infections. The gut microbiota can change the course of the disease by affecting the immune response. The overactive and insufficient immune response mediated by the gut microbiota can lead to serious clinical side effects (9). Microorganisms are the source of microorganism-related molecular models (MAMPs) and pathogen-related molecular models (PAMPs). These can be recognized by host cells through model recognition receptors (PRRs) including Toll-like receptors (TLRs) and nucleotide binding receptors (NODs). Different immunological reactions occur as a result of

TLRs' recognition of MAMPS and PAMPs and contingent on the types of cell, ligand, or receptor. This preparation of PRRs expressed in innate cells is required as a protective mechanism during secondary infection / pathogenic exposures. Short-chain fatty acids such as butyrate, acetate and propionate, which are products of the microbiota, and secondary bile acids bind to their receptors in natural cells such as dendritic cells and macrophages and regulate their metabolism and functions (1). For example, supplying of *Bifidobacterium lactis* to healthy elderly volunteers has been reported to cause a significant increase in the numbers of mononuclear leukocytes and tumoricidal activity of NK cells (10). It is known that the composition of the balanced gut microbiota has a great influence on the efficiency of lung immunity. Mice lacking gut microbiota have been shown to have low lung pathogen clearance potential. The long-term use of antibiotics increases the risk of lung cancer; there are data showing that influenza infection causes an increase in Enterobacteriaceae and a decrease in Lactobacilli and Lactococci in the intestinal microbiota (1).

Nutrition and intestinal microbiota

Nutrition plays an important role in shaping the composition of the gut microbiota (1). Factors such as systemic stress, tissue damage, and persistent inflammation can also cause acute changes in the gut microbiota. Low fat diet increases the Bifidobacterium density in stool; it has been reported that a high saturated fat diet causes an enhance in the density of *Faecalibacterium prausnitzii*. Resident microorganisms in the gut ferment indigestible carbohydrates such as dietary fiber; the produced short-chain fatty acids (acetate, propionate and butyrate) are necessary for the development of enterocytes and the regulation of the immune system (11). Prebiotic compounds such as inulin, polydextrose, corn fiber can improve immunity, intestinal diversity, digestion. It has been reported that undigested carbohydrates cause a decrease in the proinflammatory cytokine IL-6 level, a decrease in insulin resistance, and an increase in the plasma levels of the anti-inflammatory cytokine IL10 (1). The beneficial effects of prebiotics are mostly due to short-chain fatty acid production and strengthening of gastro-intestinal associated lymphoid tissue (GALT) (1). It has shown that a diet rich in fiber can not only alter the gut microbiota, but can also affect the lung microbiota. This indicates the effect of nutrition on lung immunity (12). Probiotics containing living beneficial microorganisms also have great benefits on host health. Usually Lactobacillus and Bifidobacterium strains are used as probiotics (such as *L. johnsonii*, *L. fermentum*, *L. reuteri*, *B. longum*, *B. breve*, *B. bifidum*) (1). Fermented foods like dairy products and yogurt are rich in probiotics (1). In addition to improving inflammatory conditions, probiotics are also useful in regulating innate immunity through TLRs and corresponding signaling pathways (1).

As a result, there is communication between the gut and the lung. Diet-mediated modulation of the gut microbiota may affect the lung microbiota and immune system responses. Therefore, personalized diet and microbiota-based prophylaxis can be carefully applied according to the intestinal profile in patients affected by Covid-19 to accelerate recovery and improve clinical outcomes. Studies investigating bacteria, fungi and phages in the intestinal flora and the lung microbiota of patients with Covid-19 are needed.

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APPROACH TO AVULSION CASES IN PERMANENT TEETH

Tuğba YİĞİT

Avulsion of permanent teeth is seen in 0.5%–16% of all dental injuries. (1,2) Numerous studies have shown that this injury is one of the most serious dental injuries, and the prognosis is very much dependent on the actions taken at the place of accident and promptly following the avulsion.3-17 Replantation is, in most situations, the treatment of choice but cannot always be carried out immediately. Appropriate emergency management and a treatment plan are important for a good prognosis. There are also individual situations when replantation is not indicated (eg, severe caries or periodontal disease, an uncooperative patient, severe cognitive impairment requiring sedation, severe medical conditions such as immunosuppression, and severe cardiac conditions) which must be dealt with individually. Although replantation may save the tooth, it is important to realize that some of the replanted teeth have low probability of long-term survival and may be lost or condemned to extraction at a later stage. However, not replanting a tooth is an irreversible decision and therefore saving it should be attempted. In this regard, a recent study has shown that replanted teeth have higher chances of long-term survival after following the IADT treatment guidelines, compared to previous studies.(18)

Fırst aıd for avul sed teeth at the place of accıdent

Dentists should be prepared to give appropriate advice to the public about first aid for avulsed teeth. (2,11,19-27) An avulsed permanent tooth is one of the few real emergency situations in dentistry. In addition to increasing the public awareness by mass media campaigns or other means of communication, parents, guardians and teachers should receive information on how to proceed following these severe and unexpected injuries. Also, instructions may be given by telephone to people at the emergency site. Immediate replantation of the avulsed tooth is the best treatment at the place of the accident. If for some reason this cannot be carried out, there are alternatives such as using different types of storage media. If a tooth is avulsed, make sure it is a permanent tooth (primary teeth should not be replanted) and follow these recommended instructions:

1. Keep the patient calm.

2. Find the tooth and pick it up by the crown (the white part). Avoid touching the root. Attempt to place it back immediately into the jaw.

3. If the tooth is dirty, rinse it gently in milk, saline or in the patient's saliva and replant or return it to its original position in the jaw. (28,29)

4. It is important to encourage the patient/guardian/teacher/other person to replant the tooth immediately at the emergency site.

5. Once the tooth has been returned to its original position in the jaw, the patient should bite on gauze, a handkerchief or a napkin to hold it in place.

6. If replantation at the accident site is not possible, or for other reasons when replantation of the avulsed tooth is not feasible (eg, an unconscious patient), place the tooth, as soon as possible, in a storage or transport medium that is immediately available at the emergency site.

This should be done quickly to avoid dehydration of the root surface, which starts to happen in a matter of a few minutes. In descending order of preference, milk, HBSS, saliva (after spitting into a glass for instance), or saline are suitable and convenient storage mediums. Although water is a poor medium, it is better than leaving the tooth to air-dry. (28,29)

7. The tooth can then be brought with the patient to the emergency clinic.

8. See a dentist or dental professional immediately

Treatment Guıdelınes For Avulsed Permanent Teeth

The choice of treatment is related to the maturity of the root (open or closed apex) and the condition of the periodontal ligament (PDL) cells. The condition of the PDL cells is dependent on the time out of the mouth and on the storage medium in which the avulsed tooth was kept. Minimizing the dry time is critical for survival of the PDL cells. After an extra-alveolar dry time of 30 minutes, most PDL cells are non-viable. (30,31) For this reason, information regarding the dry time of the tooth prior to replantation or prior to being placed in a storage medium is very important to obtain as part of the history. From a clinical point of view, it is important for the clinician to assess the condition of the PDL cells by classifying the avulsed tooth into one of the following three groups before commencing treatment:

1. The PDL cells are most likely viable. The tooth has been replanted immediately or within a very short time (about 15 minutes) at the place of accident.

2. The PDL cells may be viable but compromised. The tooth has been kept in a storage medium (eg, milk, HBSS (Save-a-Tooth or similar product), saliva, or saline, and the total extra-oral dry time has been <60 minutes).

3. The PDL cells are likely to be non-viable. The total extra-oral dry time has been more than 60 minutes, regardless of the tooth having been stored in a medium or not.

These three groups provide guidance to the dentist on the prognosis of the tooth. Although exceptions to the prognosis do occur, the treatment will not change, but may guide the dentist's treatment decisions.

Treatment guidelines for avulsed permanent teeth with a closed apex

<u>1.1. The tooth has been replanted at the site of injury or before the patient's arrival at the dental clinic</u>

1. Clean the injured area with water, saline, or chlorhexidine.

2. Verify the correct position of the replanted tooth both clinically and radiographically.

3. Leave the tooth/teeth in place (except where the tooth is malpositioned; the malpositioning needs to be corrected using slight digital pressure).

4. Administer local anesthesia, if necessary, and preferably with no vasoconstrictor.

5. If the tooth or teeth were replanted in the wrong socket or rotated, consider repositioning the tooth/teeth into the proper location up to 48 hours after the traumatic incident.

6. Stabilize the tooth for 2 weeks using a passive flexible splint such as wire of a diameter up to 0.016" or 0.4 mm32 bonded to the tooth and adjacent teeth. Keep the composite and bonding agents away from the gingival tissues and proximal areas. Alternatively, nylon fishing line (0.13-0.25 mm) can be used to create a flexible splint, using composite to bond it to the teeth. Nylon (fishing line) splints are not recommended for children when there are only a few permanent teeth for stabilization of the traumatized tooth. This stage of development may result in loosening or loss of the splint. (33) In cases of associated alveolar or jawbone fracture, a more rigid splint is indicated and should be left in place for about 4 weeks.

7. Suture gingival lacerations, if present.

8. Initiate root canal treatment within 2 weeks after replantation

9. Administer systemic antibiotics.(34,35)

10. Check tetanus status.(36)

11. Provide post-operative instructions.

12. Follow up.

1.2. The tooth has been kept in a physiologic storage medium or stored in non-physiologic conditions, with the extra-oral dry time less than 60 minutes

Physiologic storage media include tissue culture media and cell transport media. Examples of osmolality-balanced media are milk and Hanks' Balanced Salt Solution (HBSS).

1. If there is visible contamination, rinse the root surface with a stream of saline or osmolality-balanced media to remove gross debris.

2. Check the avulsed tooth for surface debris. Remove any debris by gently agitating it in the storage medium. Alternatively, a stream of saline can be used to briefly rinse its surface.

3. Put or leave the tooth in a storage medium while taking a history, examining the patient clinically and radiographically, and preparing the patient for the replantation.

4. Administer local anesthesia, preferably without a vasoconstrictor. (37)

5. Irrigate the socket with sterile saline.

6. Examine the alveolar socket. If there is a fracture of the socket wall, reposition the fractured fragment into its original position with a suitable instrument.

7. Removal of the coagulum with a saline stream may allow better repositioning of the tooth.
8. Replant the tooth slowly with slight digital pressure. Excessive force should not be used to replant the tooth back into its original position.

9. Verify the correct position of the replanted tooth both clinically and radiographically.

10. Stabilize the tooth for 2 weeks using a passive, flexible wire of a diameter up to 0.016" or 0.4 mm. (32) Keep the composite and bonding agents away from the gingival tissues and proximal areas. Alternatively, nylon fishing line (0.13-0.25 mm) can be used to create a flexible

splint, using composite to bond it to the teeth. Nylon (fishing line) splints are not recommended for children when there are only a few permanent teeth as stabilization of the traumatized tooth may not be guaranteed. In cases of associated alveolar or jawbone fracture, a more rigid splint is indicated and should be left in place for about 4 weeks.

11. Suture gingival lacerations, if present.

12. Initiate root canal treatment within 2 weeks after replantation (refer to "Endodontic Considerations").38,39

13. Administer systemic antibiotics. (34,35)

14. Check tetanus status. (36)

15. Provide post-operative instructions.

16. Follow up. (see: "Follow-up procedures")

1.3. Extra-oral dry time longer than 60 minutes

1. Remove loose debris and visible contamination by agitating the tooth in physiologic storage medium, or with gauze soaked in saline. Tooth may be left in storage medium while taking a history, examining the patient clinically and radiographically, and preparing the patient for the replantation.

2. Administer local anesthesia, preferably without vasoconstrictor.

3. Irrigate the socket with sterile saline.

4. Examine the alveolar socket. Remove coagulum if necessary. If there is a fracture of the socket wall, reposition the fractured fragment with a suitable instrument.

5. Replant the tooth slowly with slight digital pressure. The tooth should not be forced back to place.

6. Verify the correct position of the replanted tooth both clinically and radiographically.

7. Stabilize the tooth for 2 weeks (40) using a passive flexible wire of a diameter up to 0.016" or 0.4 mm. (32) Keep the composite and bonding agents away from the gingival tissues and proximal areas. Alternatively, nylon fishing line (0.13-0.25 mm) can be used to create a flexible splint, with composite to bond it to the teeth. A more rigid splint is indicated in cases of alveolar or jawbone fracture and should be left in place for about 4 weeks.

8. Suture gingival lacerations, if present.

9. Root canal treatment should be carried out within 2 weeks

10. Administer systemic antibiotics. (34,35)

11. Check tetanus status. (36)

12. Provide post-operative instructions. (see: "Post-operative instructions")

13. Follow up.

Delayed replantation has a poor long-term prognosis. (41) The periodontal ligament becomes necrotic and is not expected to regenerate. The expected outcome is ankylosis-related (replacement) root resorption. The goal of replantation in these cases is to restore, at least temporarily, esthetics and function while maintaining alveolar bone contour, width, and height. Therefore, the decision to replant a permanent tooth is almost always the correct decision even if the extra-oral dry time is more than 60 minutes. Replantation will keep future treatment options open. The tooth can always be extracted, if needed, and at the appropriate point following prompt inter-disciplinary assessment. Parents of pediatric patients should be informed that decoronation or other procedures such as autotransplantation might be necessary later if the replanted tooth becomes ankylosed and infra-positioned, depending on the patient's growth rate (41-46) and the likelihood of eventual tooth loss. The rate of ankylosis and resorption varies considerably and can be unpredictable.

Treatment guidelines for avulsed permanent teeth with an open apex

2.1. The tooth has been replanted before the patient's arrival at the clinic

1. Clean the area with water, saline, or chlorhexidine.

2. Verify the correct position of the replanted tooth both clinically and radiographically.

3. Leave the tooth in the jaw (except where the tooth is malpositioned; the malpositioning needs to be corrected using slight digital pressure).

4. Administer local anesthesia, if necessary, and preferably with no vasoconstrictor.

5. If the tooth or teeth were replanted in the wrong socket or rotated, consider repositioning the tooth/teeth into the proper location for up to 48 hours after the trauma.

6. Stabilize the tooth for 2 weeks using a passive and flexible wire of a diameter up to 0.016” or 0.4 mm. (32) Short immature teeth may require a longer splinting time. (47) Keep the composite and bonding agents away from the gingival tissues and proximal areas. Alternatively, nylon fishing line (0.13-0.25 mm) can be used to create a flexible splint, using composite to bond it to the teeth. In cases of associated alveolar or jawbone fracture, a more rigid splint is indicated and should be left in place for 4 weeks.

7. Suture gingival lacerations, if present.

8. Pulp revascularization, which can lead to further root development, is the goal when replanting immature teeth in children. The risk of external infection-related (inflammatory) root resorption should be weighed against the chances of revascularization. Such resorption is very rapid in children. If spontaneous revascularization does not occur, apexification, pulp revitalization/ revascularization, (48,49) or root canal treatment should be initiated as soon as pulp necrosis and infection is identified

9. Administer systemic antibiotics. (34,35)

10. Check tetanus status. (36)

11. Provide post-operative instructions.

12. Follow up.

In immature teeth with open apices, there is a potential for spontaneous healing to occur in the form of new connective tissue with a vascular supply. This allows continued root development and maturation. Hence, endodontic treatment should not be initiated unless there are definite signs of pulp necrosis and infection of the root canal system at follow-up appointments.

2.2. The tooth has been kept in a physiologic storage medium or stored in non-physiologic conditions, and the extra-oral time has been less than 60 minutes Examples of physiologic or osmolality-balanced media are milk and HBSS.

1. Check the avulsed tooth and remove debris from its surface by gently agitating it in the storage medium. Alternatively, a stream of sterile saline or a physiologic medium can be used to rinse its surface.

2. Place or leave the tooth in a storage medium while taking the history, examining the patient clinically and radiographically and preparing the patient for the replantation.

3. Administer local anesthesia, preferably without vasoconstrictor.

4. Irrigate the socket with sterile saline.

5. Examine the alveolar socket. Remove coagulum, if necessary. If there is a fracture of the socket wall, reposition the fractured segment with a suitable instrument.

6. Replant the tooth slowly with slight digital pressure.

7. Verify the correct position of the replanted tooth both clinically and radiographically.

8. Stabilize the tooth for 2 weeks using a passive and flexible wire of a diameter up to 0.016" or 0.4 mm. (32) Keep the composite and bonding agents away from the gingival tissues and proximal areas. Alternatively, nylon fishing line (0.13-0.25 mm) can be used to create a flexible splint, with composite to bond it to the teeth. In cases of associated alveolar or jawbone fracture, a more rigid splint is indicated and should be left for about 4 weeks.

9. Suture gingival lacerations, if present.

10. Revascularization of the pulp space, which can lead to further root development, is the goal when replanting immature teeth in children. The risk of external infection-related (inflammatory) root resorption should be weighed against the chances of revascularization. Such resorption is very rapid in children. If spontaneous revascularization does not occur, apexification, pulp revitalization/revascularization, (48,49) or root canal treatment should be initiated as soon as pulp necrosis and infection is identified (refer to Endodontic Considerations). 11. Administer systemic antibiotics. (34,35)

12. Check tetanus status. (36)

13. Provide post-operative instructions.

14. Follow up.

2.3. Extra-oral time longer than 60 minutes

1. Check the avulsed tooth and remove debris from its surface by gently agitating it in the storage medium. Alternatively, a stream of saline can be used to rinse its surface.

2. Place or leave the tooth in a storage medium while taking the history, examining the patient clinically and radiographically and preparing the patient for the replantation.

3. Administer local anesthesia, preferably with no vasoconstrictor.

4. Irrigate the socket with sterile saline.

5. Examine the alveolar socket. If there is a fracture of the socket wall, reposition the fractured segment with a suitable instrument.

6. Replant the tooth slowly with slight digital pressure.

7. Verify the correct position of the replanted tooth both clinically and radiographically.

8. Stabilize the tooth for 2 weeks using a passive and flexible wire of a diameter up to 0.016" or 0.4 mm. (32) Keep the composite and bonding agents away from the gingival tissues and proximal areas. Alternatively, nylon fishing line (0.13-0.25 mm) can be used to create a flexible splint, with composite to bond it to the teeth. In cases of associated alveolar or jawbone fracture, a more rigid splint is indicated and should be left for about 4 weeks.

9. Suture gingival lacerations, if present.

10. Revascularization of the pulp space, which can lead to further root development and maturation, is the goal when replanting immature teeth in children. The risk of external infection-related (inflammatory) root resorption should be weighed against the chances of revascularization. Such resorption is very rapid in children. If spontaneous revascularization does not occur, apexification, pulp revitalization/revascularization, or root canal treatment should be initiated as soon as pulp necrosis and infection is identified

11. Administer systemic antibiotics. (34,35)

12. Check tetanus status. (36)

13. Provide post-operative instructions.

14. Follow up.

Delayed replantation has a poor long-term prognosis. (41) The periodontal ligament becomes necrotic and is not expected to regenerate. The expected outcome is ankylosis-related (replacement) root resorption. The goal of replantation in these cases is to restore esthetics and function, at least temporarily, while maintaining alveolar bone contour, width and height. Therefore, the decision to replant a tooth is almost always the correct decision even if the extra-oral time is more than 60 minutes. Replantation will keep future treatment options open. The tooth can always be extracted later if needed, and at the appropriate point following a prompt inter-disciplinary assessment. Parents should be informed that decoronation or other procedures such as autotransplantation might be necessary if the replanted tooth becomes ankylosed and infra-positioned depending on the patient's growth (41-46) and the likelihood of tooth loss. The rate of ankylosis and resorption varies considerably and can be unpredictable.

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STREPTOCOCCUS PNEUMONİAE AS A RESPİRATORY PATHOGENE AND PFGE METHOD IN SEROTYPİNG

1.Tutku ARSLANTAŞ, 2.Alper KARAGÖZ, 3. Nadir KOÇAK

Streptococcus pneumoniae is one of the most common causes of pneumonia, bacteraemia and meningitis . It is estimated that one to two million adults and at least one million children, mostly in developing countries, die from pneumococcal infections every year. Across Europe also, pneumococcal infections are responsible for considerable morbidity and mortality, particularly in the very young and the elderly .There is an increasing rate and spread of antibiotic resistance. S. *pneumoniae* is capable of horizontal transfer of capsule genes, virulence genes and resistance determinants, and there are reported cases of these events occurring. There is also the likelihood of pneumococci acquiring antibiotic-resistance determinants from other species . The vaccines available are against 7 or 23 capsular serotypes of the overall 90 defined serotypes. There is therefore a risk of emergence and dissemination of strains resistant to antimicrobial agents that are not covered by the vaccines. Serotyping is the traditional phenotypic method used to differentiate pneumococcal strains. Serotypes are grouped together when they share at least one epitope and produce antibodies that cross-react. Of the 90 serotypes, 65 belong to 21 different serogroups containing two to five types. Today, serotyping is the standard method used to characterize pneumococci, and the knowledge gained from the type distributions forms the basis of the currently available vaccines.

For the past two decades, a number of genotyping methods aimed to assess the genetic diversity of pneumococcal isolates have been used. Currently, pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) are the gold standards for genotyping of pneumococci.

PFGE, a genotypic method for the evaluation of total chromosomal DNA, has been considered the ‘gold standard’ when typing many micro-organisms and has been employed for typing S.*pneumoniae* .

In PFGE, total DNA is digested with a rare cutter endonuclease (such as *Sma*I or *Apa*I) that yields a limited number of fragments of high molecular weight. The fragments are separated by a variant of gel electrophoresis in which the orientations of the electric field change periodically, enabling megabase size DNA fragments to be effectively separated by size. The DNA banding patterns are then compared between isolates and clonal relationships are inferred . Although the interpretation criteria may vary depending on the size of the collection and on the goal of the research being conducted, there are general criteria, both visual and computer-assisted that seem to work well . Advantages of PFGE are that it has good typeability (the percentage of isolates that can be assigned a type), reproducibility, and resolving power. In addition, the costs for materials and equipment are relatively low and handling of the equipment is easy. However, it is laborious and time consuming and may yield ambiguous results if not performed by a well trained technician. PFGE is quite useful for local epidemiology and it has also been used for global epidemiology once standardized. Portability between laboratories is not straightforward, but seems to be attainable provided protocol harmonization is achieved.

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ANTIBIOTIC USage IN DENTISTRY

Umut YİĞİT

There are documents showing that in 2500 BC, the Chinese treated some infections with plants and fungi. In 1857, with Pasteur's discovery of the antagonism between mold and microbes, the discovery of antibiotics accelerated. After observing the effect of fungi on pathogenic microorganisms, the word antibiosis was introduced into our lives by Pasteur in 1871. However, the real antibiotic era started in 1929 with the discovery of penicillum notatum by Fleming. In parallel with the discovery of antibiotics, important developments have been made in this field with the recognition of the structure of bacteria.

The Human Microbiome Project has identified more than 70 million 16s ribosomal RNA sequences in 15 regions of the body.[1]
In this direction, it is possible to define the human body as a super organism.

Recent research shows that our microbiome plays an important role in our development and health. The oral mucosa, a complex environment containing more than 1000 phylotypes, is one of the regions where different types of microorganisms are seen together.[2] Not all of these microorganisms are considered pathogenic and most play a key role in maintaining both oral and systemic health. If this balance established between the human body and microorganisms is broken in favor of pathogens, the body becomes susceptible to infections. In order for antibiotics to show the correct efficacy without harming the host, the presence of a microbiologically proven bacterial infection must be questioned. Its selective effect, that is, its effect only on the pathogen without harming the host depends on the administration of the appropriate antibiotic in appropriate doses. Antibiotic used in appropriate dosages shows its effectiveness by damaging the vital structures such as cell walls, cytoplasm and DNA of bacteria. As in all medical branches, the use of antibiotics has an important place in dentistry.
According to the retrospective data of the Ministry of Health for the years 2011-2015, 82.4% of the drugs prescribed by dentists are antibacterial drugs. In dental practice, antibiotics are used prophylactically in odontogenic and non-odontogenic infections to prevent infection in individuals at risk, and as an aid to clinical practice in order to prevent the spread of existing local or systemic infection. Antibiotic use should not be seen as an alternative to dental treatment. Dentists often turn to empirical therapy to prescribe broad-spectrum antibiotics to manage the current situation. Although they have important efficacy, the use of broad-spectrum antibiotics is generally undesirable. [3] In cases where the indication is suspected, it is important to apply the necessary treatment by performing an antibiotic sensitivity test. Prescribing the wrong antibiotics is a serious problem all over the world. Antibiotics that are not used with the correct indication cause bacterial resistance, increased side effects and economic burdens.

When the data of the Centers for Disease Control and Prevention (CDC) of 2017 are examined, it is thought that 30% of all antibiotics prescribed in the United States are unnecessarily prescribed [4]. According to the data of the European Center for Disease Prevention and Control (ECDC) for 2018, the antibiotic consumption of the European Union member countries was determined as an average of 18.4 DID (defined daily dose per 1000 people) [5]. Turkey, according

to a report of Antimicrobial Consumption Network of the World Health Organization, the country has been seen with a maximum value of 40.4 DID consumption from 11 countries. [6] Rational use of antibiotics has been defined by the World Health Organization as the need for the prescribed antibiotic to be administered at appropriate intervals and at the right doses, the patient to access the antibiotic at low cost, and the drug to be of acceptable safety and quality.[7] Based on these findings and definition, antibiotic use should be based on accurate symptoms and diagnostic patterns. The medical history of the patient should be evaluated by considering the side effects of the drugs used and drug interactions. It should be decided to provide maximum benefit to the patient with minimum harm.

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Epitope identification and receptor design for biosensor based cancer detection

Zeynep ALTINTAŞ

The conformation of biological molecules on surfaces or in solution environments strongly effects the successful implementation of biosensing platforms for the detection of target molecules as possible conformational changes lead to decreased sensing signals. To date, artificial protein binders have been developed using linear peptides with an unknown structure in epitope imprinting process. Despite successful outcomes obtained to some extent, most of these works lack of providing either high affinity, selectivity or sensitivity.

We aim to address these problems by performing molecular dynamic calculations for the design of high affinity artificial protein binding surfaces for cancer biomarker recognition [1]. Computational simulations are employed to identify particularly stabile secondary structure elements. These epitopes are used for subsequent molecular imprinting, where surface imprinting approach is applied [1-4]. The molecular imprints generated with the calculated epitopes of greater stability show better binding properties than those of lower stability. The average binding strength of imprints created with stabile epitopes is found to be around fourfold higher for the selected biomarker models [1]. The artificial protein binders can recognise the target molecules even in a complex medium including non-specific molecules at a high concentration [1-4]. Certain amino acid modifications of the computationally selected epitope templates (e.g. addition of histidine to the peptide chain or cysteine modification on both terminal of the elongated peptide to form self-assembled monolayer bridges) further improve the performance of artificial protein binders [2, 3]. Moreover, we have fabricated dual-epitope imprinted sensors using two distinct surface exposed epitopes of target biomarker (neuron specific enonalase, NSE) in order to increase the capturing efficacy of the synthetic receptors [4]. The sensitivity of the sensor is further enhanced by adding gold nanoparticles (AuNPs) in the polymer network [4]. The fabrication of the sensors has successfully been characterized by several electrochemical and microscopic techniques [1-4]. Compared to single epitope imprints, the dual-epitope imprinted sensor decorated with AuNPs has resulted in 20 times lower limit of detection (LOD: 25 pg mL^{-1}) in human serum and allowed to detect the cancer biomarker in a wide concentration range (25–4000 pg mL^{-1}) with a very high affinity (dissociation constant: 1.54 pM). In addition, a high selectivity was established by comparing the performance of the imprinted sensors against non-imprinted polymers [4].

Our novel and rational selection can be used for establishing epitope libraries for protein molecules by eliminating unsuitable epitopes and ranking the best candidates based on their stability analysis obtained from molecular dynamic simulations. The integrated approach has shown a good potential to contribute to some limitations of medical diagnostic field. Research disciplines that require recognition receptors can apply this technique for designing stable and efficient receptors.

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Label-free DNA Sensor Applications in Detection of Pathogens

Sümeyra SAVAŞ

Nowadays, biosensors meet new technologies such as nanomaterials, and you have a fast and precise measurement connection with low cost, from proteins, pesticides, pathogens in air, water and food to antibiotics in water. With the ability of DNA immobilized to the surface to be bound to the surface with high stability, it is aimed to create a surface with high selectivity for its complement. In this study, the bacteria-specific gene we selected was used as the target, and these are synthetically produced DNA probes. With the Label-free DNA sensor developed with the support of nanoparticles, it is aimed to detect pathogens / pathogens. DNA capture probes developed against 3 different pathogens and one virus were chemically immobilized on the gold-coated sensor surface. Target DNA was bound by capture and hybridization basis and electrochemical measurement was taken by using nanoparticle instead of enzyme. In real-time measurements, different bacteria specific target concentrations were tried and the limit of detection in the DNA sensor was determined.

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Genomic and structural analysis of SARS-COV-2 and its detection using CRISPR diagnostics

Abdul Razaque MEMON

Abstract

SARS-CoV-2 (genera β-COVs-lineage B) was first identified in Wuhan, China in December 2019, is the cause of a catastrophic pandemic (severe acute respiratory syndrome), COVID-19, affecting 213 countries and territories having more than 32 million infections diagnosed worldwide by mid-September 2020. SARS-CoV-2 has the largest identified positive single-stranded RNA genome of approximately 30 kilobases (kb) in size. The genome contains a 5'-cap structure along with a 3'- poly(A) tail, allowing it to act as an mRNA for translation of the replicase polyproteins. It contains four structural and 16 non-structural proteins. Its RNA-dependent-RNA polymerase display proofreading function and is possibly responsible for the stabilization of this long RNA sequence. These proteins are expressed in two ways: primary translation of polyprotein that initiates the infection, and after some replication, subgenomic mRNA expression produces all structural proteins. Here I provide the details of the genome organization and the mechanism of virus and host cell interaction. I have also discussed in brief the replication of this virus, its pathogenicity, and diagnostic methods and treatment strategies.

Introduction

The SARS-CoV-2 is the strain of coronavirus that causes the disease COVID-19 and was first reported in Wuhan, China, in December 2019 [1](Zhou et al., 2020). SARS-CoV-2 is the seventh coronavirus known to infect humans; SARS-CoV, MERSCoV and SARS-CoV-2 can cause severe disease, whereas HKU1, NL63, OC43 and 229E are associated with mild symptoms [2]. Coronaviruses possess the largest genomes among any RNA viruses. On March 11, 2020, World Health Organization (WHO) classified COVID-19 as a pandemic, and this disease has stressed health systems and the global economy and is a severe socioeconomic challenge for most of the governments of the world, including Turkey. Genes for the major structural proteins in all coronaviruses occur in the 5'-3' order as S, E, M, and N (Fig 1.) [3].

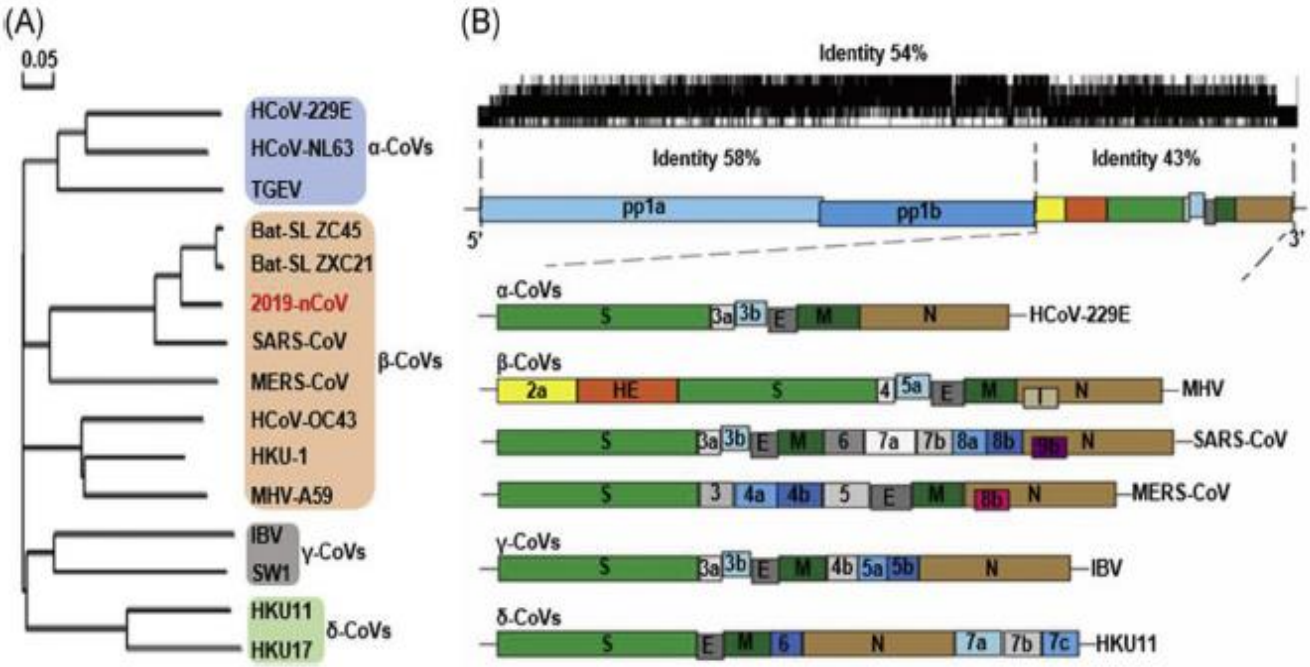


Fig. 1. The genomic structure and phylogenetic tree of coronaviruses: A, the phylogenetic tree of representative CoVs, with the new coronavirus COVID-19 shown in red. B, The genome structure of four genera of coronaviruses: two long polypeptides 16 nonstructural proteins have proceeded from Pp1a and pp1b represent. S, E, M, and N are represented of the four structural proteins spike, envelope, membrane, and nucleocapsid. COVID-19; CoVs, coronavirus; HE, hemagglutinin-esterase. Viral names: HKU, coronaviruses identified by Hong Kong University; HCoV, human coronavirus; IBV, infectious bronchitis virus; MHV, murine hepatitis virus; TGEV, transmissible gastroenteritis virus [3].

The 2019-nCoV has a round, elliptic, or pleomorphic form with a diameter of 60–140 nm. It has a positive single-stranded RNA genome of approximately 30kb (~29891 bp), the largest of RNA viruses, 5'-capped, 3'-polyadenylated and infectious. This Poly(A) tail allows coronaviruses direct translation after infection without needing an intermediate transcription stage. Transcription initiation is regulated in coronaviruses by several types of consensus transcription regulating sequences: TRS1-L, 5'-cuaaac-3', TRS2-L, 5'-acgaac-3' and merged into TRS3-L, 5'-cuaaacgaac-3'. These multiple TRS give place to several subgenomic polycistronic mRNAs, encoding structural, non-structural and accessory proteins [4]. Coronavirus genome includes multiple open-reading frames (ORF) containing genes which are transcribed by several transcription regulating sequences (TRS). Genes encoding non-structural proteins are placed at 5' UTR (ORF1.1, ORF1.2 ...) (NSP1 TO NSP16) which contribute to transcription replication, whereas at 3'-UTR are genes for structural (N, M, E and S). These genes are interspaced with several accessory genes, encoding accessory proteins (AP), characteristics in number of each virus type (Fig. 2).

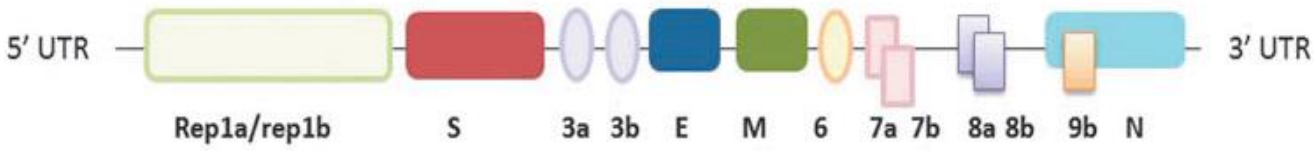


Fig 2. Structural proteins are encoded by the four structural genes, including spike (S), envelope (E), membrane (M), and nucleocapsid genes (N), and also genome organization and the encoded proteins of pp1ab and pp1a and accessory proteins (3a, 3b, 6, 7a, 7b,8a, 8b, 9b, and ORFs)[5].

SARS-CoV-2 is similar to the previously known severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS). Genetic analyses reveal that bats are possibly the natural reservoir of all major coronaviruses, including SARS-CoV-2 and other animals are likely the potential intermediate hosts for emergences of these viral diseases in humans [6].

Structure and Genome Organization

The genome of Covid-19 has been sequenced and deposited to NCBI reported from different laboratories across the world. It contains 29.811 bp longs nucleotides (cDNA) broken down as follows: 8.903 (29.86%) adenosines, 5.482 (18.39%) cytosines, 5.852 (19.63%) guanines, and 9.574 (32.12%) thymines. Updated sequence information has been provided in the Table 1 [7].

Table 1. List of genomes sequenced by different countries [7].

Accession	Number Strain/Origin
MN988668	2019-nCoV WHU01
NC_045512	Wuhan-Hu-1
MN938384.1	2019-nCoV_HKU-SZ-002a_2020
MN975262.1	2019-nCoV_HKU-SZ-005b_2020
MN988713.1	2019-nCoV/USA-IL1/2020
MN994467.1	2019-nCoV/USA-CA1/2020
MN994468.1	2019-nCoV/USA-CA2/2020
MN997409.1	2019-nCoV/USA-AZ1/2020
MN985325.1	2019-nCoV/USA-WA1/2020
MT072688	SARS0CoV-2/61-TW/human/2020/NPL
MT106054	2019/nCoV/USA-TX1/2020
MT012098.1	SARS-CoV-2/human/IND/29/2020
MT050493.1	SARS-CoV-2/human/IND/166/2020

The SARS-CoV-2 genome is similar to that of the SARS-CoV and MERS-CoV viruses, with 88 % and 50 % of sequence identity, respectively [7].

In 5'-3' sense, the genome is organized into fourteen open reading frames (ORFs) (Fig. 3a) that encode a variety of structural or non-structural proteins, according to their functions in the viral particle. It contains several essential genes that encode the viral proteins necessary for replication, transcription, and infectious virus assembly. These essential genes comprise the open reading frames 1a and 1b (ORF1ab) and corresponds to almost two thirds of the virus RNA. ORF 1a and ORF 1b sequences are translated into two large overlapping polyproteins called pp1a and pp1ab [8, 9], which are processed into sixteen non-structural proteins (nsps 1-16), many of them are involved in viral RNA replication and transcription [10, 11]. The nsps include two viral cysteine proteases, including papain-like protease (PLpro)(nsp3), chymotrypsin-like, 3C-like, or main protease (nsp5), RNA-dependent RNA polymerase (nsp12), helicase (nsp13), and others likely to be involved in the transcription and replication of SARS-CoV-2 [9, 12, 13, 14]. Cleavage occurs between the products of ORF1a and ORF1b to form pp1a made up of nsps 1–11, while pp1ab will be made up of nsps 1–16 (Fig. 3b) [11]. The other SARS-CoV-2 ORFs that correspond to one third of the viral genome, encode for the four structural proteins which are involved in infectious virus assembly: spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins [12]. The N protein holds the RNA genome, and the S, E, and M proteins together create the viral envelope. The spike protein is responsible for allowing the virus to attach to and fuse with the membrane of a host cell by utilizing its cellular angiotensin-converting enzyme 2 (ACE2). Interspersed between these genes in the coronavirus genome are several other genes called group-specific or accessory genes. They encode accessory proteins and are dispensable for virus growth *in vitro*, but may play an essential role in modulating the host response to virus infection and thereby, contribute to pathogenesis**.**

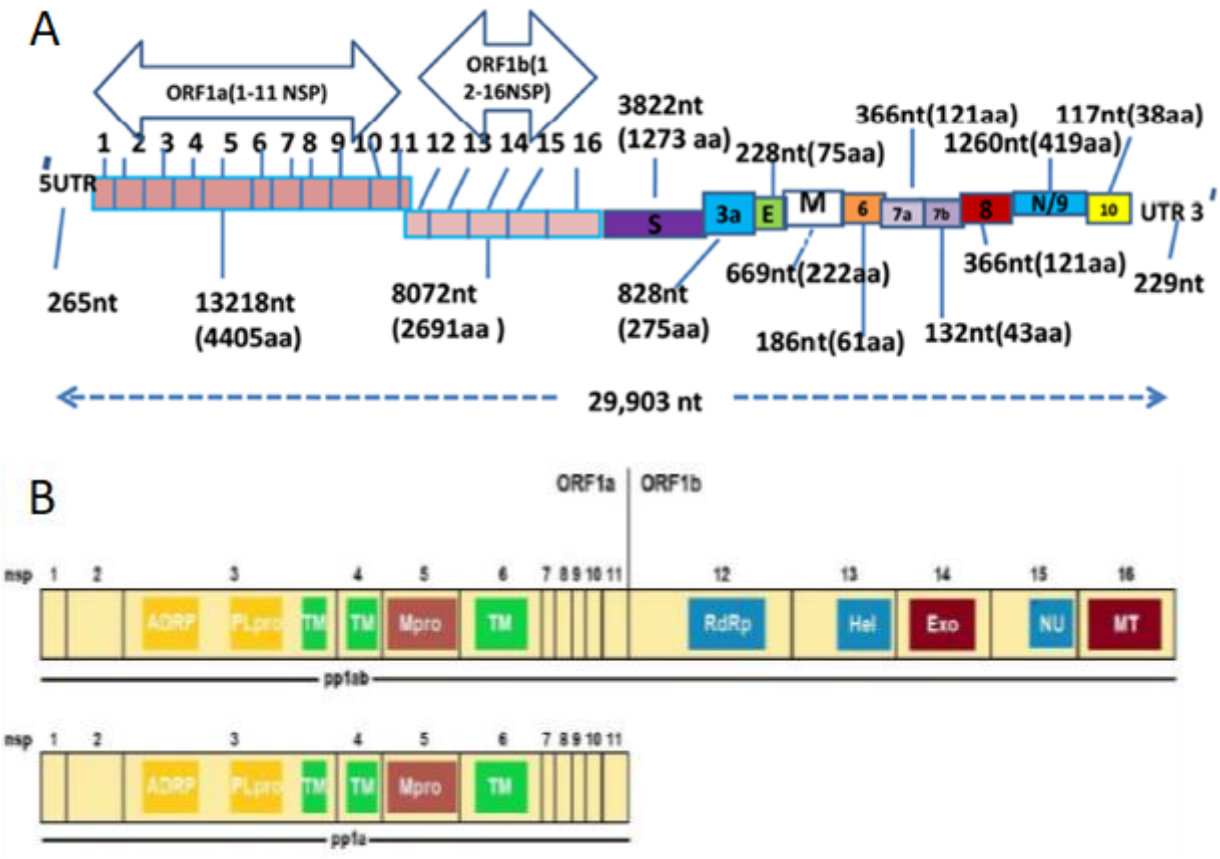


Fig. 3 -Structure of the SARS-CoV-2 virus genome. A. An overall genome +ssRNA showing different non-structural proteins(nsp1-16) and structural proteins (S-Spike, E- Envelope, M-membrane, N-nucleocapsid n and ORF genes with nucleotides(nt) and amino acid number(aa) of product proteins [9]. B- Schematic diagram of ORF1a and ORF1b. TM, transmembrane; Mpro, chymotrypsin-like protease; RdRp, RNA-dependent RNA polymerase; Hel, helicase; Exo, 3 '→ 5' exonuclease; NU, NendoU: uridylate-specific endoribonuclease; MT, ribose-2′-O-methyltransferase; nsp, non-structural protein [11].

The structure of SARS-CoV-2 was observed by electron microscopy after 3 days of post-infection in the cell. Electron microscopic view revealed the coronavirus-specific morphology of SARS-CoV-2 with virus particle sizes ranging from 70 to 90 nm observed under a wide variety of intracellular organelles, most specifically in vesicles [15]. Due to high sequence similarity, the structure of SARS-CoV-2 is speculated to be the same as SARS-CoV [16]. The surface viral protein spike, membrane, and envelope of coronavirus are embedded in host membrane-derived lipid bilayer encapsulating the helical nucleo-capsid comprising viral RNA (Fig.4) [17]. The structure of spike [18] and protease of SARS-CoV-2 [19] have been resolved, which provides an opportunity to develop a newer class of drugs for the treatment of COVID-19.

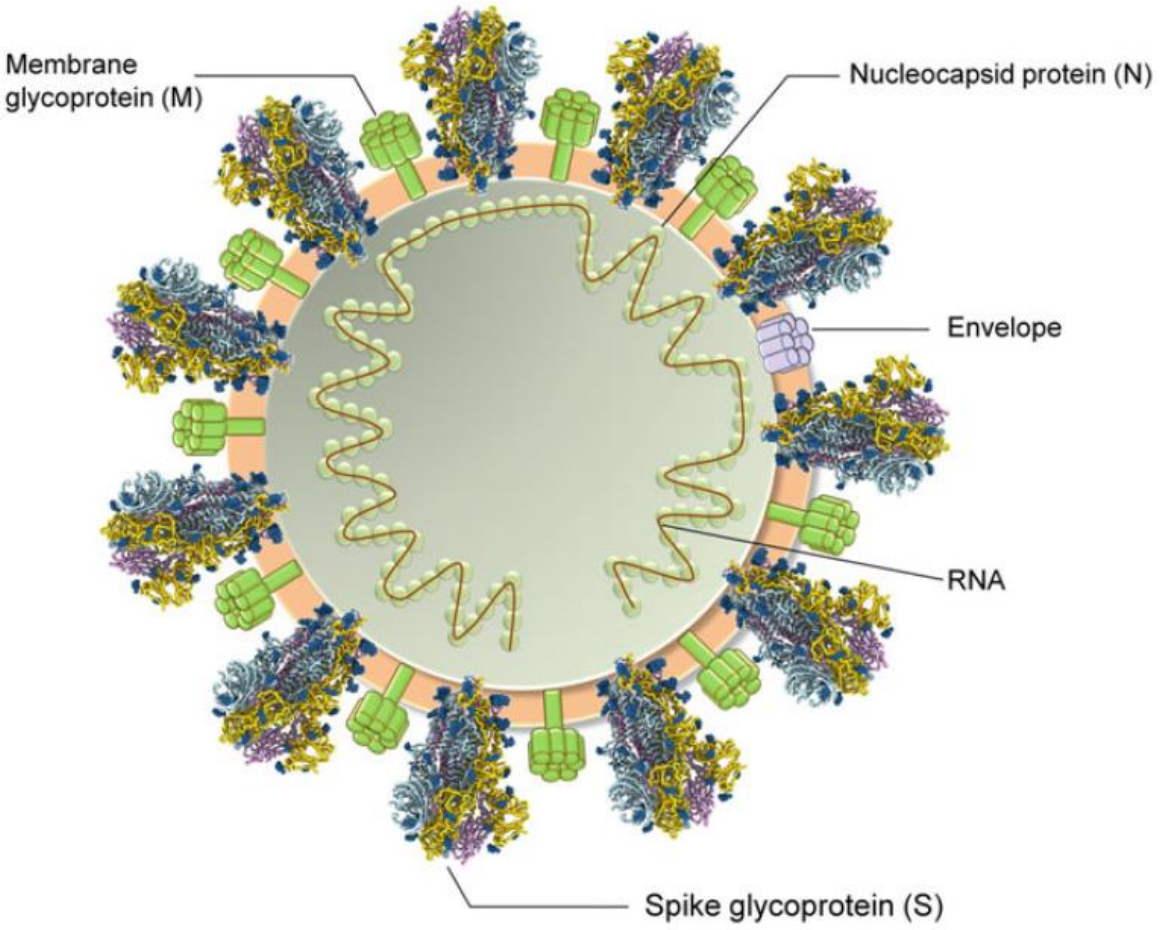


Fig.4. Structure of SARS-CoV-2. SARS-CoV-2 has viral surface proteins, namely, spike glycoprotein (S), which mediates interaction with cell surface receptor ACE2. The viral membrane glycoprotein (M) and envelope (E) of SARS-CoV-2 are embedded in host membrane-derived lipid bilayer encapsulating the helical nucleo-capsid comprising viral RNA [16].

Mutations in SARS-Cov-2

Coronaviruses have genetic proofreading mechanisms and SARS-CoV-2 sequence diversity is very low [20]. Still, natural selection can act upon rare but favorable mutations. Five mutations have been identified, including T8782C (in ORF1a, codons AGT to AGC, silent mutation), T9561C (in ORF1a, codons TTA to TCA, non-silent mutation), C15607T (in ORF1b, codons CTA to TTA, silent mutation), C28144T (in ORF8b, codons TCA to TTA, non-silent mutation), and T29095C (in Nucleocapsid, codons TTT to TTC, silent mutation) [21]. In a few studies, it was shown that mutation in SARS CoV-2 was present at nucleotide level in gene S, nsp1, nsp3 and nsp15 but not at amino acid level [22]. In addition, the SARS-CoV-2 strain found in the US, the Nucleocapsid (N) protein gene has three mutations (28881G>A, 28882G>A, and 28883G>C). Studies have revealed that the N protein of SARS-CoV is responsible for the formation of the helical structure during virion assembly.

The N protein has potential value in vaccine development because it may cause immune response. Also, mutations associated with spike glycoprotein have been found. The significant SNP mutation (23403A>G) occurred in the gene encoding spike glycoprotein (S protein: D614G). The mutation D614G is located in the putative S1–S2 junction region near the furin

recognition site (R667) for the cleavage of S protein when the virus enters or exits cells [23]. Korber et al. 2020 showed that SARS-CoV-2 variant carrying the Spike protein amino acid change D614G has become the most prevalent form in the global pandemic. They have presented an evidence that virus containing G614 form of mutation in spike protein globally circulating in human population compared to the original virus strain first identified in Wuhan, China (D614). Their Follow-up studies showed that patients infected with
G614 shed more viral nucleic acid compared with those with D614, and G614-bearing viruses show significantly higher infectious titers in vitro than their D614 counterparts [24].
Spike glycoprotein structure was predicted in SARS-CoV-2 (Fig. 4) and two conformations, closed and open deduced via cryo-electron microscopy deposited to the protein data bank with pdb id 6VXX and 6VYB, respectively. It has been suggested that SARS-CoV polyclonal antibodies may inhibit SARS-CoV-2 spike mediated entry into cells [25]. It has been found that the S1 domain of spike protein mediates an initial high-affinity association with their ACE2 receptor [26]. Walls et al and Zhou et al. have demonstrated experimentally that SARS-CoV-2 uses ACE2 (human angiotensinconverting enzyme 2) to enter inside the target cell and shows a similar affinity towards ACE2 as SARS-CoV [27]. Consequently, the spike protein has been considered a viral target. The Mechanisms of viral entry may include membrane fusion upon receptor binding and induced conformational changes in spike protein followed by cathepsin L (CTSL) proteolysis which leads to activation of membrane fusion within endosomes [28].

SARS-CoV-2 Non-Structural Proteins

SARS-CoV-2 non-structural proteins play a crucial role in several processes in the virus and in the host cells. The functions of these proteins are summarized in Table 2 and Fig 5. Briefly, nsp 1 promote inhibition of type 1 interferon (IFN) signaling and block the innate immune response in the host cell [29]. The nsp2 binds to a proinhibitory protein while nsp3 and nsp5 promote the expression of cytokines [30].The nsps 4 and 6 contribute to the structure of the double membrane vesicles [31,32], nsp7/8 is a hexadecameric complex that support of the replication enzyme and nsp8 constitutes a second RNA polymerase that can function as a primase [33]. RNA-dependent RNA polymerase is represented by nsp12, which together with the helicase RNA (nsp13) guarantee the assembly of the replicase transcriptase complex [34]. Nsp9 constitutes an RNA-binding protein phosphatase, which like nsp10, is part of the replicase complex. nsp10 constitute a necessary link for the adequate function of the main viral protease (Mpro) [30]. Nsp14 exhibits exonuclease activity 3'→5' with a role in maintaining the fidelity of RNA transcription [35], and (guanine-N7)-methyl transferase activity, involved in RNA cap formation [36]. Nsp16 is involved in RNA cap formation [37]. Finally, nsp15 encode a uridylate-specific endoribonuclease (NendoU) that is crucial for virus replication and distinguishes nidoviruses from other RNA viruses [38].

Table 2. Nonstructural proteins of coronaviruses and their function [11, 30].

Non-structural proteins	**Function**
nsp1	Inhibition of type 1 IFN signaling; block of the innate immune response in the host cell.
nsp2	Binds to a proinhibitory protein of unknown function.
nsp3	PLpro activity for processing the pp1a and pp1ab polyproteins; cytokine expression; IFN-β antagonist; deubiquitinase activity.
nsp4	Double membrane vesicles formation.

nsp5	3CLpro activity for processing of pp1a and pp1ab polyproteins; cytokine expression.
nsp6	Double membrane vesicles formation.
nsp7	Hexadecameric complex formation.
nsp8	Primase; hexadecameric complex formation.
nsp9	RNA binding protein phosphatase.
nsp10	Part of the replicase complex.
nsp11	Unknown.
nsp12	RNA-dependent RNA polymerase.
nsp13	RNA Helicase.
nsp14	3'→5' exonuclease activity; N7-MTase activity for the RNA cap formation.
nsp15	Uridylate specific endoribonuclease.
nsp16	2'O-MTase activity for the RNA cap formation.

PLpro, papain-like proteases; IFN, Interferon; 3CLpro, chymotrypsin-like protease Mpro; N7-MTase, (guanine-N7)-methyltransferase; 2'O-MTase, nucleoside-2'O-methyl transferase. Figure 5 presents a scheme of ORF1.1 gene and non-structural proteins (nsp1 to nsp16), including accessory protein AP2.

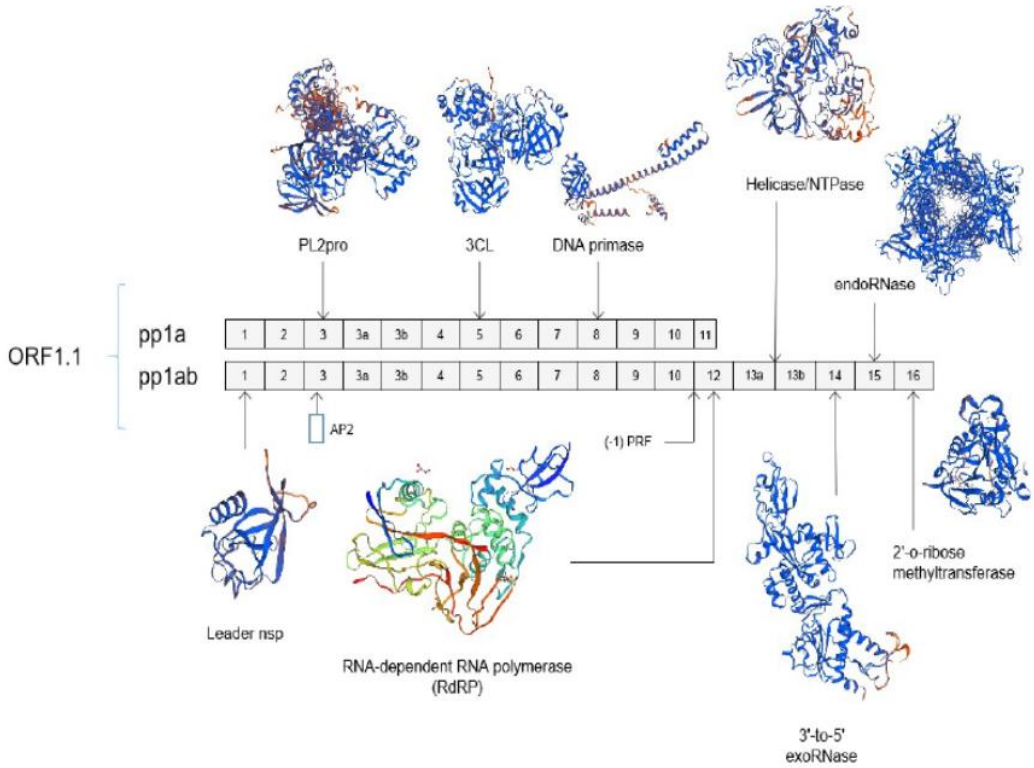


Fig. 5 – Scheme of the ORF1.1 gene and description of the non-structural proteins (nsp1 to nsp16) of SARS-CoV (SWISS Model). Accessory protein AP2 has been included [39].

SARS-CoV-2 RNA-dependent RNA Polymerase

SARS-CoV-2 uses an RNA-dependent RNA polymerase (RdRp) for the replication of its genome and the transcription of its genes [40, 41]. Hillen et al present a cryo-electron microscopy structure of the SARS-CoV-2 RdRp in an active form that mimics the replicating enzyme [42](Fig 6). This structure is composed of comprises viral non-structural protein 12 (nsp12), nsp8 and nsp7, and more than two turns of RNA template–product duplex (Fig. 6). This polymerase makes a new RNA strand using RNA, not DNA, as the template. So, the virus simply needs to encode this polymerase in its RNA genome. Then, the polymerase is made by cellular ribosomes soon after infection and it starts replicating the viral RNA to make new viruses. Since human cells don't make RNA from RNA, this polymerase is an attractive target for antivirals. New drugs usually take a long time to perfect since they have to be evaluated for effectiveness and safety, but investigational polymerase-targeting agents like remdesivir are being repurposed in the fight against COVID19. Remdesivir was developed to fight hepatitis and Ebola, but wasn't very effective. It is currently approved for emergency use against SARS-CoV-2.

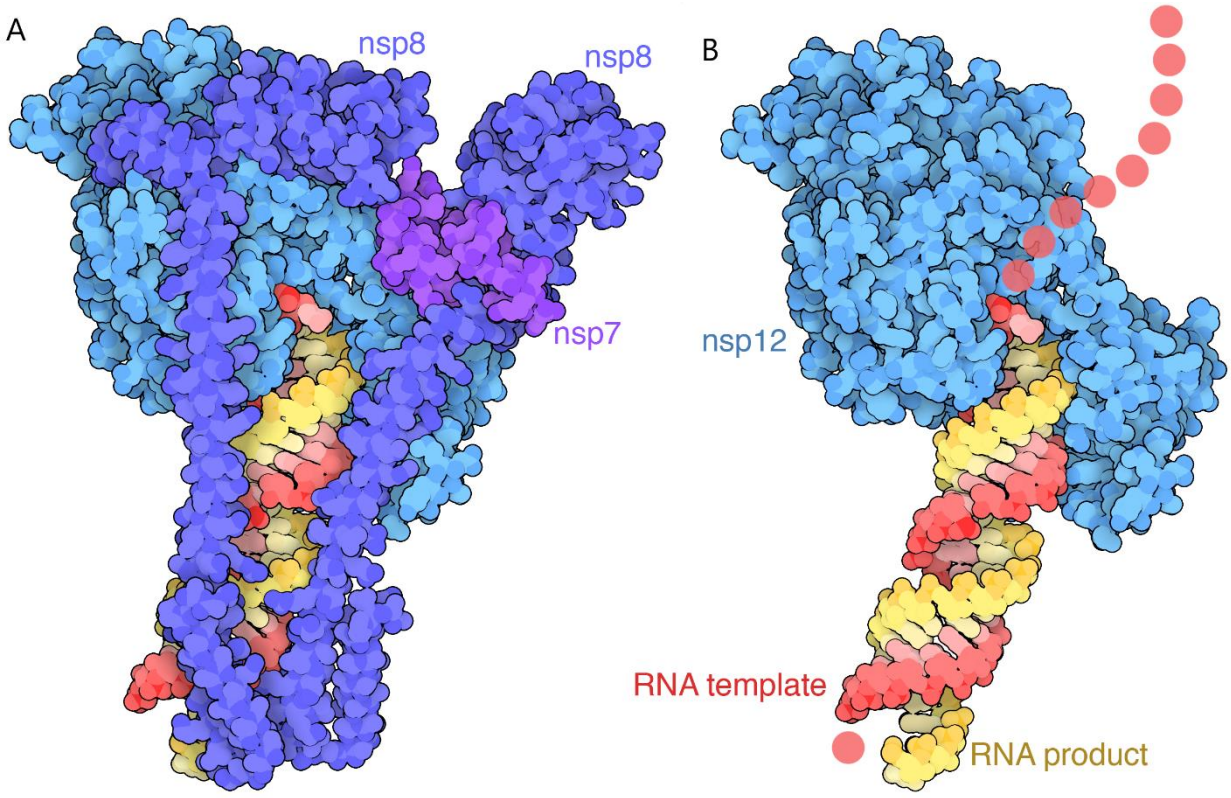


Fig. 6. A. SARS-CoV-2 RNA-directed RNA polymerase (nsp12) with nsp7 and nsp8, and a short duplex of RNA with a template strand and a product strand. B. Nsp7 and 8 are removed on the right to show the interaction with RNA [42].

The RdRp of SARS-CoV-2 is composed of a catalytic subunit known as nsp12 (Fig. 6 B) as well as two accessory subunits, nsp8 and nsp7 (Fig 6. A). Nsp 7 and 8 assist the polymerase in its job, but they are also thought to help get the whole process started by creating a primer at the start of the RNA chain (Fig 6 A, B). This structure shows a large, circular complex of SARS-CoV nsp7 and nsp8 composed of 16 chains [42]. Researchers have speculated that it encircles

the RNA strand and creates the primer. It is currently unknown if something similar occurs with SARS-CoV-2.

SARS-CoV-2 Structural Proteins

The SARS-CoV-2 virus contains four main structural proteins, including spike (S), membrane (M), nucleocapsid (N), and envelope (E) proteins, which are encoded inside the 3' end of the viral genome**.**

Spike (S) virus glycoprotein is a densely glycosylated trimer and is 16-21 nm long spicules (38) which bind to host receptor ACE2. It is class I viral protein cleaved by a furin-like protease into two equal size peptides S1 and S2. The cleavage site between the two subunits (amino acids 682-685, RRAR) creates a polybasic furin site which enhance the virus transmission in the human cells through endocytosis. In this infection process, S1 peptide regulates the virus-host interaction and S2 facilitates the membrane fusion and viral entry into the host cells (Fig 7) [43]. Membrane proteins (M) is the most abundant structural protein involved in shaping the viron integration in the host cell. This protein is monomer ranges from 25 to 30 KDa and is embedded in the virus envelope through three transmembrane domains[44]. Envelope (E) protein is a small polypeptide that is found in limited amounts in the viral envelope which facilitates assembly, envelope formation, and budding. It is abundantly expressed within the infected cell during the replication cycle but only a small amount is incorporated into the envelope of the virion. Large amount of this protein is located in the Golgi complex, where it take parts in the assembly of the particle and is very important in the production and maturation of the viral particle [45].

Nucleocaspid (N) protein contains two domains that allow it to recognize viral RNA and binds to nsp3. It plays a critical role in encapsidated genome packaging into virons and is effective in viral replication [46]. This protein also participates in the repression of host cell interference RNAs that suppress the expression of specific sequences of the viral genome which constitute a vital part of the body's immune response to viruses [47].

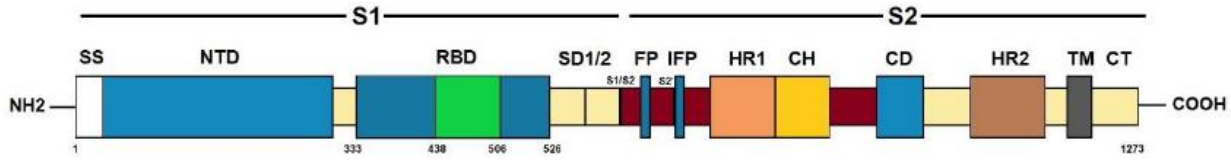


Fig. 7. Schematic diagram of the SARS-CoV-2 S protein.

Primary structure of S protein. SS, signal sequence; NTD, N-terminal domain; RBD, receptor-binding domain, RBM, receptor-binding motif; SD1/2, subdomain 1 & 2; S1/S2 and S2', protease cleavage site; FP, fusion peptide; IFP, internal fusion peptide; HR1, heptad repeat 1; CH, central helix; CD, connector domain; HR2, heptad repeat 2; TM, transmembrane domain; CT, cytoplasmic tail [14].

Spike-ACE 2 Interaction

The new study focused on mutations to a key part of SARS-CoV-2 – its "spike protein." This protein binds to a protein on human cells called ACE2, a necessary step for infection (Fig 8). Mutations in the spike protein could change how well SARS-CoV-2 sticks to – and thus infects – human cells**.** Starr et al.2020 displayed a fragment of the spike protein (receptor binding domain) and this fragment made direct contact with ACE2 in bread yeast cells [48]. They systematically change every amino acid in the receptor binding domain (RBD) of the SARS-CoV-2 spike protein and created thousands of versions of the fragment – each with different

mutations. That let them assess how various mutations might affect the function of the binding domain. This work identified structurally constrained regions of the spike RBD that would be ideal targets for COVID-19 countermeasures and demonstrated that the mutations in the virus that enhance ACE2 affinity can be engineered but have not, to date, been naturally selected during the pandemic**.**

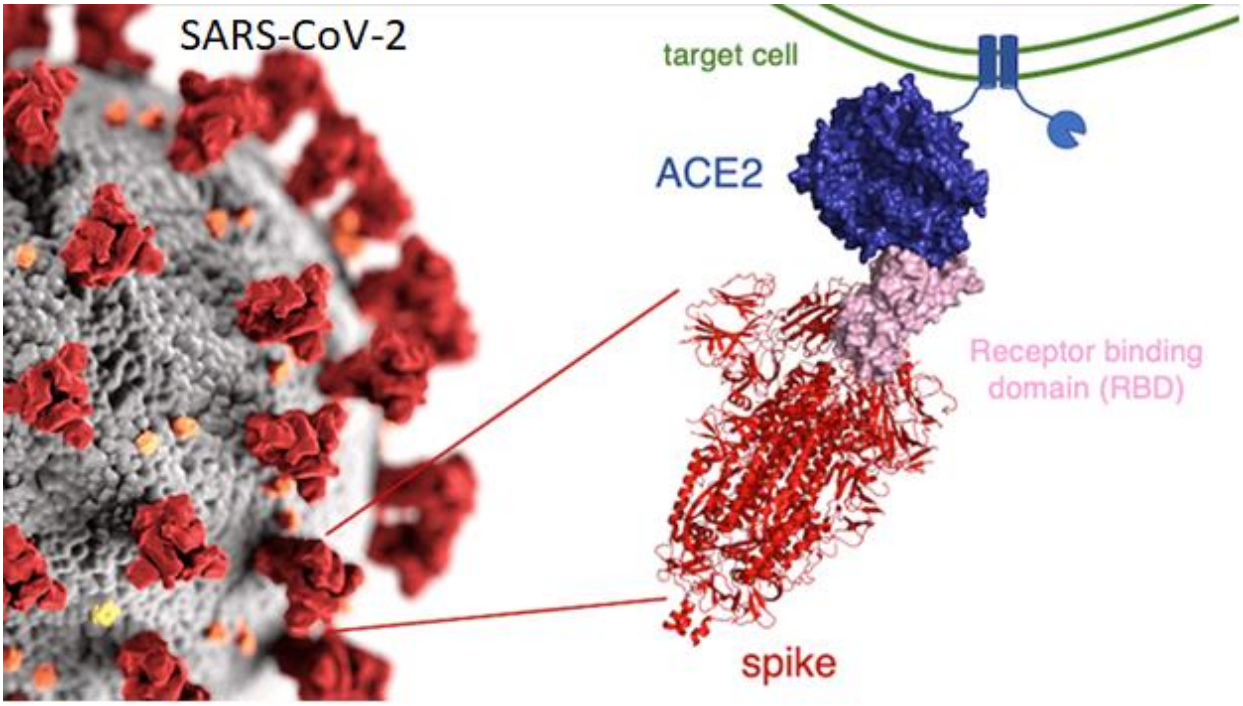


Fig. 8. Researchers are studying mutations to the part of SARS-CoV-2 (red and pink) that makes contact with the ACE2 protein (blue) on human cells. The work could reveal how the virus's ability to infect cells may change over time. Credit: Tyler Starr/Bloom Lab and Alissa Eckert/MSMI; Dan Higgins/MAMS.

The author suggested that this data will be valuable for researchers designing drugs and vaccines to fight COVID-19. Understanding the consequences of different mutations can guide the development of drugs that will continue to work as the virus changes over time. They proposed that the antibodies which stick to this part of the virus are really good and protective antibodies that would elicit with a vaccine.

Replication of Virus in the Cell

SARS-CoV-2 primarily infects ciliated bronchial epithelial cells and type II pneumocytes, where it binds to the surface receptor, angiotensin-converting enzyme 2 (ACE2), through S glycoprotein found on its surface (Fig. 9) [49]. When S glycoprotein binds to the ACE2, the cleavage of trimer S protein is triggered by the cell surface-associated transmembrane protease serine 2 (TMPRSS2) and cathepsin. S glycoprotein includes two subunits, S1 and S2. S1 determines the host range and cellular tropism and facilitates viral attachment to the target cells. S2 is a unit that mediates the fusion of viral and cellular membranes, ensuring viral entry through endocytosis [49]. The affinity between the virus's surface proteins and its receptors is a critical step for viral entry. A recent study showed that the affinity between S glycoprotein of SARS-CoV-2 and ACE2 binding efficiency is 10–20 fold higher than that of SARS-CoV, which could explain the highly infectious ability of SARS-CoV-2 [50].

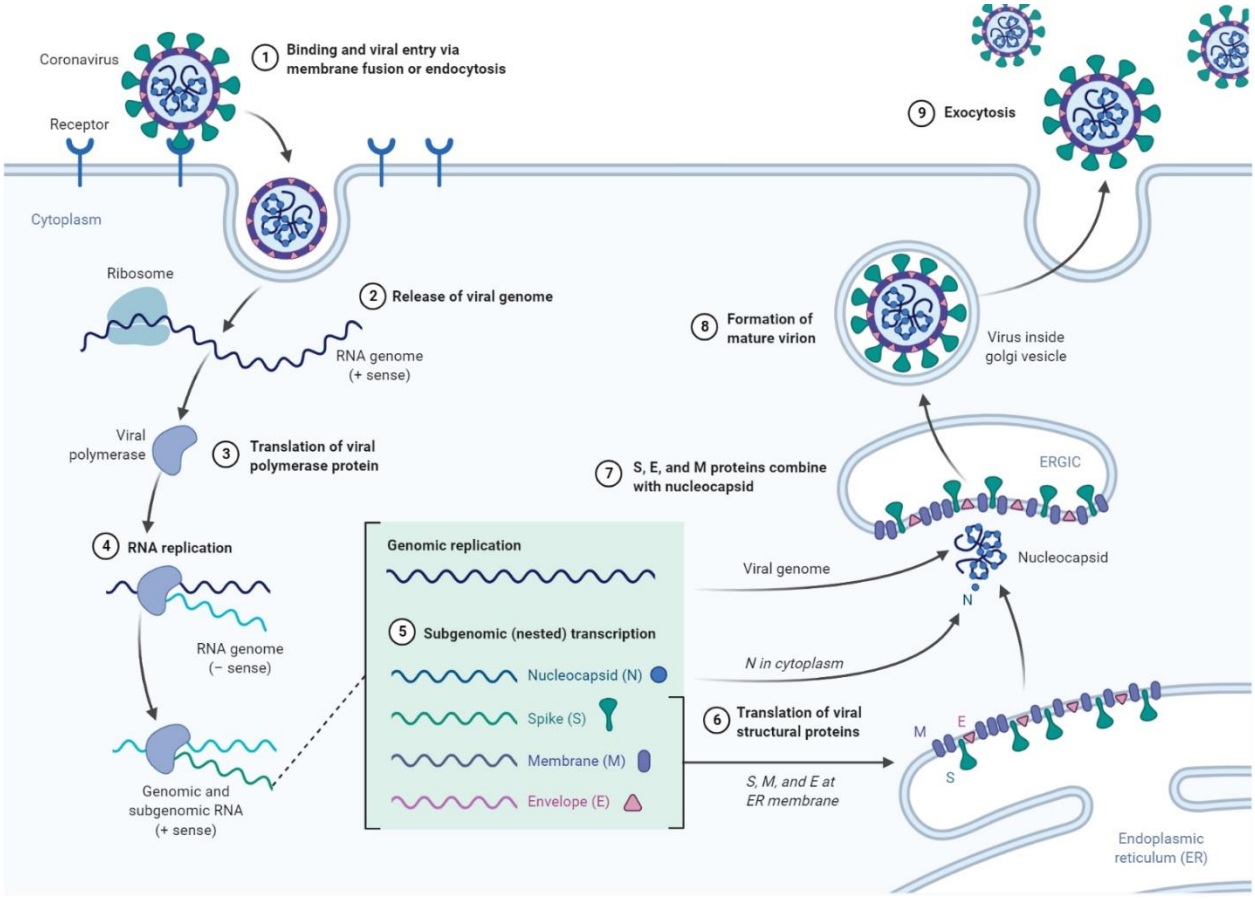


Fig.9. The life cycle of SARS-CoV-2 in the host cells. Scheme of a Coronavirus cycle replication: (1) Attachment and fusion (APN/ACE2/DPP4), (2) endocytosis, (3) translation of vRNA (ORF1.1), (4) assembly of RNA-dependent RNA polymerase (RdRp) and other non-structural proteins (nsp) by proteases, (5) transcription of subgenomic mRNAs by RdRp, (6) translation of subgenomic mRNAs and protein synthesis, (7) assembly into membraneous regions ERGIC and (8) fusion with plasma membrane and (9) exocytosis (BioRender http://app.biorender.io) [51].

CRISPR/Cas as a Potential Diagnosis Technique for COVID-19

Recently COVID-19 cases are persistently increasing in various countries (WHO 2020) and it is important to diagnose the disease as early as possible for patient treatment and recovery. It is becoming urgent to find out or to develop new diagnostic techeniques which could be rapid, cost-effective and easy to use. Aditionally, it is very important to understand that how silent infections that are in the presymptomatic or asymptomatic phase contribute to the transmission of the COVID-19 to the population. The effectiveness of symptom-based interventions depends on the fraction of infections that are asymptomatic, the infectiousness of those asymptomatic cases, and the duration and infectiousness of the presymptomatic phase. It has been reported that individuals during their presymptomatic phase could be in the most infectious category of the

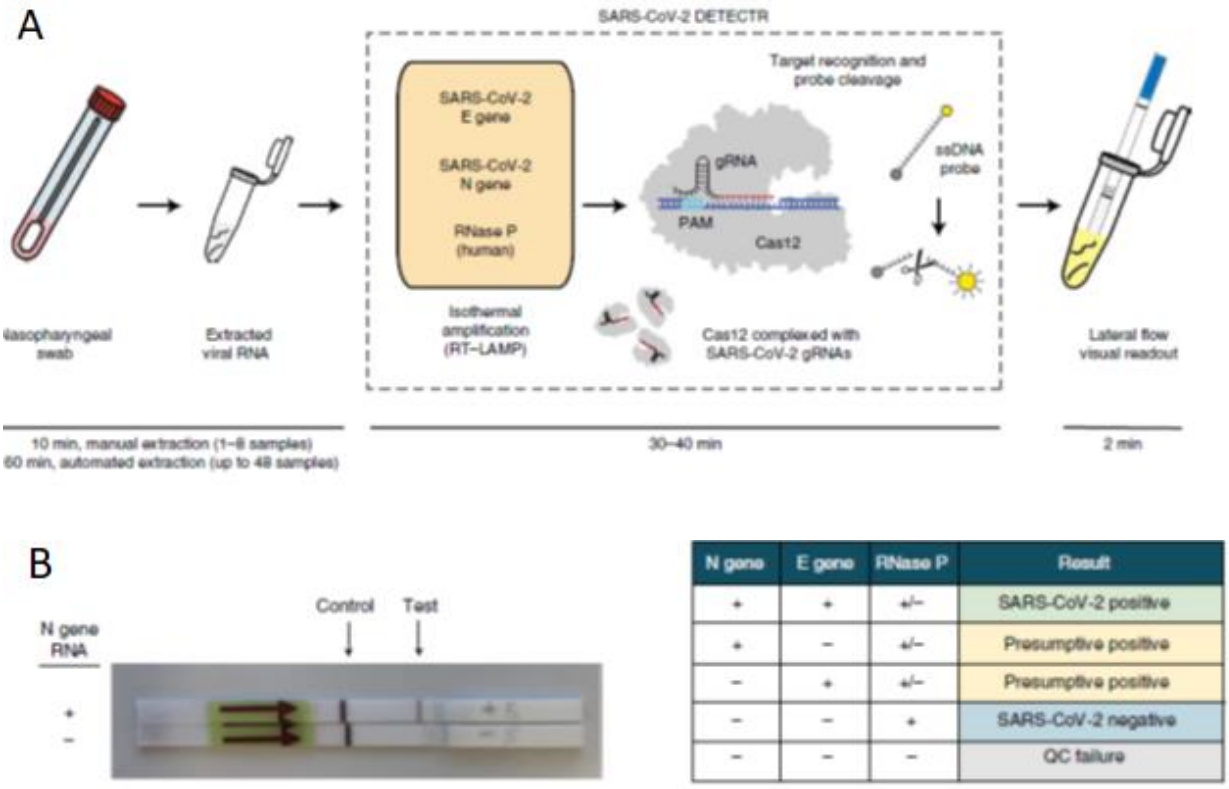
pandemic [52]. Moghadas et al repoted that the silent disease transmission during the presymptomatic and asymptomatic stages are responsible for more than 50% of the overall attack rate in COVID-19 outbreaks [53]. Even if all symptomatic cases are immediately isolated, the silent transmission alone can sustain outbreaks in uninfected population. There is an urgent need to scale up testing of suspected cases without symptoms as noted in revised

guidelines by Centers for Disease Control and Prevention (CDCP 2020)(Centers for Disease Control and Prevention, Overview of testing for SARS-CoV-2. https://www.cdc.gov/coronavirus/2019-ncov/hcp/testing-overview.html?CDC_AA_refVal= https%3A%2F%2Fwww.cdc.gov%2Fcoronavirus%2F2019-ncov%2Fhcp%2Fclinical-criteria.html#changes, Accessed 20 June 2020).

The increase in demand for rapid screening and identification of COVID-19 poses great diagnostic challenges. Next-generation sequencing (mNGS) and reverse-transcription PCR (RT-PCR) are the most commonly used molecular methods in the major clinical laborotries for diagnosing COVID-19, but these methods have their own limitations. For example, sequencing is costly and nearly takes one full day for obtaining the results, while RT-PCR requires a specific equipment (real time PCR machine) and is difficult for low income countries to deploy at a large scale. Due to these limitations and the lack of rapid, accurate and cost-effective molecular diagnostic tools has hampered efficient public health responses to the viral threat. In such a backdrop, any development toward ultrasensitive, cheaper, and portable diagnostic tests for the assessment of suspected cases, regardless of the presence of qualified personnel or sophisticated equipment for virus detection, could help advance the diagnosis of COVID-19.

CRISPR is a genomic technique well-known for its use in genome editing. Recently, CRISPR technique has been employed for the *in vitro* detection of nucleic acids and is emerged as a powerful and precise tool for molecular diagnosis [54]. Within the CRISPR-Cas effector family, Cas12 is a RNA-guided DNase belonging to the class II type V-A system that induces indiscriminate single-stranded DNA (ssDNA) collateral cleavage after target recognition. This leads to the degradation of ssDNA reporters that, emit a fluorescence signal on cleavage or alternatively, could be detected on a paper strip (by lateral flow) in a portable manner. Therefore, CRISPR-Cas12 based tools possess the potential to emerge as an *in situ* diagnostic tool for rapid detection of the SARS-CoV-2 virus. Recently several research groups have developed a new low-cost diagnostic test based on CRISPR/cas 12 for COVID-19 which quickly delivers accurate results without the need for sophisticated equipment [55, 56]. Broughton et al have reported a CRISPR-based DETECTR (DNA Endonuclease-Targeted CRISPR Trans Reporter) assay which provides a visual and faster alternative to the US Centers for Disease Control and Prevention SARS-CoV-2 real-time RT–PCR assay, with 95% positive predictive agreement and 100% negative predictive agreement [55]. In this assay both reverse transcription and isothermal amplification using loop-mediated amplification are peformed simultaneously for RNA

extracted from nasopharyngeal or oropharyngeal swabs in universal transport medium (UTM), followed by Cas12 detection of predefined coronavirus sequences, after which cleavage of a reporter molecule confirms detection of the virus [55]. The procedure in brief is illustreated in Fig. 10. A and B.



N gene	E gene	RNase P	Result
+	+	+/–	SARS-CoV-2 positive
+	–	+/–	Presumptive positive
–	+	+/–	Presumptive positive
–	–	+	SARS-CoV-2 negative
–	–	–	QC failure

Fig. 10. A CRISPR–Cas12-based assay for detection of SARS-CoV-2. A. Schematic of SARS-CoV-2 DETECTR workflow. Conventional RNA extraction can be used as an input to DETECTR (LAMP preamplification and Cas12-based detection for E gene, N gene and RNase P), which is visualized by a fluorescent reader or lateral flow strip. B**.** Lateral flow strip assay readout. A positive result requires detection of at least one of the two SARS-CoV-2 viral gene targets (N gene or E gene, as indicated in the interpretation matrix). QC, quality control [55].

Several methods for COVID-19 detection using CRISPR-Cas technology have been developed by different research groups and companies. The companies like Mammoth Biosciences, Sherlock Biosciences and CASPR Biotech are currently putting substantial time and resources in this diagnostic research inorder to commercialize their respective CRISPR based diagnostic assays. Recently the US Food and Drug Administration's (FDA) emergency-use authority has approved a CRISPR-based diagnostic kit developed by Sherlock Biosciences to allay the COVID-19 detection backlogs [57]. Similarly, the Institue of Genomics and Integrative Biology, a component lab if Indian Council of Scientific and Industrial Research and TATA Sons of India have signed an MoU to develop and license a CRISPR based FNCAS9 Editor Linked Uniform Detection Assay (FELLUDA) to provide affordable mass testing to the communities in orderly manner [58].

Conclusion

In this review I have described in some detail about the genomic structure of the SARS-CoV-2 virus and the characteristics of the structural and non-structural proteins caters the basis for understanding the mechanisms of COVID-19 infection and to develop the strategies for effective therapeutics. Vast resources are being directed towards understanding this new disease and the virus that caused it. This review paper outlines the current state of our understanding the SARS-CoV-2 infection and the newly developed cost-effective diagnostic techniques by several laboratories in different countries.

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Using of bee pollen powder to breeder japanese quails diets and effects on hatching egg quality characteristics and hatching results.

Yüksel AKIN[1], Mehmet Fatih ÇELEN[2]

Poultry meat and egg are animal protein sources that have an important effect on nourishment and antibiotics have been used as growth factors in poultry for many years because of their growth enhancement effects in poultry. However, antibiotics, as well as the pathogenic microorganisms residing in the digestive tract of animals, prevent the proliferation of beneficial microorganisms. Turkey and European Union countries, the long-term use of antibiotics as feed additives has been banned against the risk of creating microorganism strains with high antibiotic resistance in animals as well as in humans consuming these products (1-7). As a result of these developments; prebiotics, probiotics, essential oils, humates, and medicinal and aromatic plants are used as alternative feed additives against antibiotics because they prevent sub-clinical infections, encourage growth and are not harmful, but rather benefical, to human health (8-15). Bee pollen, which can be used as an alternative to antibiotics, is defined as the flower powder collected by bees. The polen values of the plant species and varieties from which the pollen is collected are increasing in terms of human health (16). For example, acer (*Acer spp*.), walnut (*Juglans regia*), mulberry (*Morus spp*.), ash (*Fraxinus spp*.), hazelnut (*Corylus spp*.), birch (*Betula spp*.), elm (*Ulmus spp.*), chestnut (*Castanea sativa*), alder (*Alnus spp*.), willow (*Salix spp*.), and boxwood (*Buxus spp*.) are important tree species from which the pollen is collected (17). Bee pollen is rich in proteins, carbohydrates and lipids, as well as the major and minor elements found in plant tissues. In addition it contains organic substances, such as amino acids, nucleic acids, enzymes, vitamins, minerals and hormones (18-22). In addition, bee pollen contain many chemical compounds for example; phytochemicals, carotenoids (color pigments), flavonoids (plant compounds with strong antioxidants) and phytosterols [plant sterols (organic matter consisting of carbon atoms)].(23). The chemical contents of bee pollen have been shown in table 1. (24).

Table 1. The chemical contents of bee pollen (24)

Components	Values	Components	Values
Energy	2,46 kcal/g	Copper	14 ppm
Protein	% 23,7	Nickel	4,5 ppm
Carbonhydrate	% 27	Thiamine	9,4 ppm
Lipid	% 4,8	Niacin	157 ppm
Phosphorus	% 0,53	Riboflavin	18,6 ppm
Potassium	% 0,58	Pantothenate	28 ppm
Calcium	% 0,225	Pyridoxine	9 ppm
Magnesium	% 0,148	Folic Acid	5,2 ppm
Sodium	% 0,044	Biotin	0,32 ppm
Iron	140 ppm	Carotenes	95 ppm
Manganese	100 ppm	Vitamin E	14 ppm
Zinc	78 ppm	Vitamin C	350 ppm

Bee pollen and pollen products contain significant amounts of polyphenol compounds with antioxidant potential. They are used in alternative medicine, treatment of some diseases and nourishment, because they have several properties, such as antimicrobial, antibacterial, anti-

allergic etc., can regulate brain functions and metabolism, and are effective against stress and psychological problems (19, 25-29).

Many researches have been done on the use of bee products in poultry breeding. Some studies in which the propolis ethanol extract and pollen were added to the Japanese quail rations have been specified not statistically significant differences ($P>0.05$) in the live weight gain (LWG) and feed conversion ratio (FCR) (30-31). If in other studies on broilers and Japanese quails, LWG and FCR values were reported to be statistically significant ($P<0.05$ and $P<0.01$) (32-34). The emergence of such differences with researches might be attributed to the age and origin of the animals used and the area and content of the pollen collected, etc. As a result of the studies on quails, it was reported that an average-sized quail egg weighed between 9.00 and 13.00 g (35). In the researches bee pollen addition on rations showed a favorable effect on the egg weight of the quails, but it is determined no statistically significant differences. The pigments xanthophylls and carotenoids provide yellow color which is one of the internal quality characteristics of eggs, and the natural and synthetic products are currently used for coloring eggs. Bee pollen can be evaluated as a good natural coloring agent because it contains carotenoids in its structure. The yellow color of eggs is an important criterion for their marketing, and it has been reported that the RCF (DSM) value generally preferred by consumers is 10 (36). The coloring effect provided by the feeds begins from after the 2nd day, and then, contiunes rising, reaching its highest levels after the 9th-12th days, as explained by (37). In the one of researches is reported RCF values obtained at at the end of week 13 were statistically significant in all BPP groups ($P <0.01$), with the highest values obtained at the end of the experiment ($P<0.01$). The researcher has been determined at the end of the experiment, it was found that the addition of BPP at different concentrations to quail rations- positively affected the RCF values; an increase in the RCF values was directly proportional to an increase in the concentration of BPP (31).

The shape index values obtained for the Japanese quails determine some internal and external quality characteristics of the quail eggs (38). In the one of studies BBP-supplemented to rations increased statistically higher yolk index values ($P<0.05$) while the white index values, which are an important egg quality criterion, not shown any significant. (31). The Haugh unit values are many important as egg quailities for poultry breeders. In many researhes, the Haugh unit values have been reported ranged between 68,47 and 88,93 in Japanese quails (31, 39-40 Many previous studies suggested that an ideal shell thickness should be at least 0.33 mm in order to be resistant to breakage, but it has been emphasized that this value should range between 0.16- and 0.23 mm in quail (41-43). BPP-supplemented has been reported not effects on quail diets rations as a statistically significant in the shell thickness, in the researches determined the average shell thickness was found to lie between 0.181 and 0.196 mm. (31, 39, 44). It has been reported that the shell that protects the egg by creating an outer layer constitutes approximately 11-12% of the egg weight, and the average shell weight of the standard quail egg is approximately 1-1.2 g (39). In the one of studies, it has been reported BPP-supplemented has not been effects on quail egg shell weight, but the BPP-supplemented groups, shell weight was found to be higher than control group probably because the bee pollen contains certain important minerals, such as calcium, potassium and phosphorus (31).

It has been reported that the ideal incubation results in chickens are associated with the loss of weight (as water vapor) in an egg (ELW) during the incubation period; this loss of weight is directly related to the number of pores in an egg shell. It has been reported, based on these criteria, the eggs were found to have lost an average weight of 11.5% during incubation in chickens and that BPP- supplemented to rations not effect ELW (31, 45-47). In previous studies,

egg weight has been shown to have significant effects on incubation. It was reported that the fertility ratio was 72.57% in the eggs weighing 7.01- 8.90 g and 83.24% in the eggs weighing 10.01-11.00 g, and the fertile hatchability was 74.08% in the eggs weighing 10.01-11.00 g, 84.28% in the eggs weighing 11.01-12.00 g (48). It has been reported in the one of researches BPP-supplemented to rations in quail diets significant fertility ratio and fertile hatchability values were observed compared to not BPP diets. ($P<0.05$). In addition, in the study the hatching performance has been found to be similar among all groups, and no significant difference has been observed in the newly hatched chick numbers ($P>0.05$). (31). In Japanese quails, the newly hatched chick weight is 6.69-8.03 g, accounting for 66.9% of the ideal average weight of a hatching egg (10-12 g). It has been specified BPP-supplemented to rations is been effective on chick weights (8.04) (31, 49). For succesful hatching; early-term deaths (ETD), middle-term deaths (MTD), late-term deaths (LTD) and external pip ratio (EPR) values are important criteria of the incubation results. It has been reported, in a research BPP-supplemented to rations, effect as positive on the ETD and EPR values, but it has been effected no statistically significant on the LTD values (31).

When examining the studies on the use of bee pollen in poultry farming, the following evaluation can be made briefly: BPP supplementation of the Japanese quail diet could be used in quail rations because it has an enhancement effect on certain egg quality characteristics, such as natural coloring, fertility ratio, fertile hatchability, shell thickness.

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ESTABLISHMENT OF AN EFFICIENT *IN-VITRO* PROTOCOL FOR CALLUS INDUCTION AND SOMATIC EMBRYOGENESIS OF LOCAL DATE PALM (*Phoenix dactylifera* L.) CULTIVARS IN PAKISTAN

Dr. Liaquat Ali Bhutto[1], Abdul Jabbar Memon[1], Khalid Iqbal Rajput[1], Dr. Jamil Ahmed Jokhio[1], Shazia Yasin Rajput[1] and Shahmeer Ali Tunio[1]

Abstract:

An efficient protocol for callus formation and somatic embryogenesis was optimized in two local date palm varieties Aseel and Karbalain at Plant Tissue Culture Laboratory, Plant Disease Research Institute, Tandojam, Sindh, Pakistan. For callus and somatic embryogenesis the MS media was used with the combination of two different auxin 2, 4-D (15, 25, 50, 73, 100 mg/l) NAA (20, 40, 80 mg/l) and cytokinin BA 4.5 mg/l. The maximum response of culture was observed in combination of 25mg/l 2, 4-D, 20mg/l NAA and 4.5mg/l BA. Moreover, 1.5g/l activated charcoal was added in MS basal medium, when the explant was taken from shoots tip. This treatment was found to induce earliest callus formation (130 days) as compared to other treatments. However higher concentration of auxin was found to aggravate growth of the callus and maximum weight of callus as compared to low concentration of auxin. The quality of callus was also affected differently with all combinations. The embryonic calli were developed after 6 months of callus which was sub-cultured on fresh MS basal medium without hormone for regeneration.

Keywords: Date Palm, Callus, Somatic Embryogenesis, Auxin, Cytokinin.

Introduction:

Date palm (*Phoenix dactylifera* L.) belongs to family Arecaceae, the origin of date palm is mainly to middle east and north Africa. Its cultivation spread to Australia, South Africa, and America (Chao and Krueger 2007) during last century. In Pakistan date palm is cultivated throughout the country due to its ability to tolerate adverse environmental conditions; the arid climate of Upper Sindh, Southern Punjab and Baluchistan is quite suitable for the cultivation of date palm. Pakistan rank 6th among dates producing countries. It is cultivated over an area of 99,032 hectares with annual production of 420,127 tones (Ministry of National Food Security Government of Pakistan 2018-19).

Date palm production is declined due to current environmental conditions. There are several reasons to hamper the production of date palm, such as major pests and diseases, salinity, drought, poor harvest and post-harvest practices (Al Khayri 2005; Jian 2001, 2011, 2012; Johnsan 2011). Congenitally date palm is propagated through offshoots or suckers. While seeds cannot be used for the propagation of commercially date palm cultivars due to their heterozygous nature which produces off type plant population. A date palm tree produces only 20-30 suckers in their whole life span.

Tissue culture technology is an alternate solution to overcome this problem, which has high potential to produce maximum number of plants in limited time and space. Which are true to type and agronomically equal or superior to conventionally propagated plants. A numbers of reports so far have been published in this regard (Tisserat 1982; Zaid *et al.* 2011 Sharma *et al.* 1986; Mater 1987; Hassan *et al.* 2012; Naik *et al.* 2016). Tissue culture is a recent technique mainly used for rapid propagation of several perennial fruit trees including date palm. Normally, date palm is propagated *in-vitro* by two methods: the first method is by embryogenesis in which vegetative embryos can continuously be formed from embryogenic callus. The second procedure is organogenesis; which produces date palm buds that eventually gives plantlets without passing through the callus stage.

Present study was focused to establish the protocols to induce the callus in short duration with good quality and best regeneration capacity through the different concentrations of growth hormones in the basal media.

Materials and Methods:

The present study was carried out at Plant Tissue Culture Laboratory, Plant Disease Research Institute, Agriculture Research Center Tandojam, Sindh, Pakistan.

Explant source:

Two different local date palm varieties (Aseel and Karbalain) were taken from the field of Date Palm Research Institute Kotdigi, Sindh, Pakistan.

Explant preparation and surface sterilization:

For the explants, the suckers were taken from mother plants during the month of September. Shoot tip was excised carefully elimination of leaves. These explants were surface sterilized with 70% ethanol for a minute then dipped into 10% commercial bleach with 1-2 drop tween-20 for 10-15 minutes. After that the explants were washed with sterilized double distilled water with several times (6-7).

Explant culturing:

Shoot tip was excised into 1.5cm and inoculated into MS medium (Murashige and Skoog 1962) supplemented with different concentration of 2,4-D (0, 15, 25, 75 and 100 mg^{-1}) NAA (20, 40, 80, mg^{-l} and 4.5mg^{-l} BA with 1.5 g/l activated charcoal.

Culture condition:

All culture bottles were kept into incubator till callus was formed. After callus formation the cultures were placed into continuous light at 2000-2200 lux for somatic embryogenesis, maturity germination and shoot proliferation at 27 ± 2^{o}C temperature.

Results and Discussions:

Effect of 2, 4-D, NAA and BA on callus induction:

The data presented in **Table. 1** showed the average days taken for callus induction in two local date palm varieties i-e; Aseel and Karbalain. Callus was not induced without growth hormones in both the cultivars. The minimum days 130 and 131 for callus induction were observed in both the cultivars in T3 and maximum days 225 and 230 for callus induction were observed in both the cultivars in T5. The pronounced effect of different combinations of Dichlorophenoxy Acetic Acid (2, 4-D), Naphthalenacetic Acid (NAA) and Benzyl Adenine (BA) was clearly induced callus in date palm cultivars. The mechanism of auxin and cytokinin mediated morphogenesis has been observed as the subject of extensive studies on differentiation of tissue and organs in various plant species.

Table 1: Effect of 2, 4-D, NAA and BA on callus induction in two local date palm cultivars.

Treatment		**Concentrations of 2,4-D and /NAA along with BA 4.5mg/l**	**Average number of days**	
			Aseel	**Karbalain**
T1	Control	0.0 + 0.0	-	-
T2	NAA +2,4-D	20+15	142	145
T3	NAA +2,4-D	20+25	130	131
T4	NAA +2,4-D	20+75	152	155
T5	NAA + 2,4-D	20+100	225	230
T6	NAA + 2,4-D	40+15	156	158
T7	NAA + 2,4-D	40+25	165	169
T8	NAA +2,4-D	40+75	170	178
T9	NAA +2,4-D	40+100	199	201
T10	NAA +2,4-D	80+15	175	180
T11	NAA + 2,4-D	80+25	160	170
T12	NAA + 2,4-D	80+75	187	189
T13	NAA + 2,4-D	80+100	190	192

Effect of 2, 4-D, NAA and BA on periodical response of culture in Aseel and Karbalain:

Table. 2 showed the periodic response of callus, growth and differentiation in both the cultivars i-e; Aseel and Karbalain. Callus induction in date palm is influenced by different parameters such as genotypes, explant types, induction period and plant growth regulators. The good quality callus was observed in T3. Meanwhile with the high concentration of 2,4-D and NAA along with BA effected the quality of callus and turned brown in color and no growth was recorded. In every sub-culturing several changes like tissue browning, cell enlargement, cell differentiation and callus initiation were observed under different treatments. In case of either shoot tips high concentrations of auxin have been used to induce embryogenic calli. 2, 4-D is reportedly the most effective auxin for embryogenic callus induction in date palm, and it has been used mainly at the concentration of 100 mg/l. Embryogenic callus induction using 100 mg/l 2, 4-D was reported by Eshraghi *et al.* (2005), Al-Khayri (2010) and Al-Khayri (2011) in many date palm cultivars. However, Fki *et al.* (2011) mentioned that high doses of 2, 4-D may induce soma clonal variation. Therefore, other researchers used lower 2, 4-D concentrations or other auxins in order to induce somatic embryogenesis. For instance, El Hadrami *et al.* (1995) used 5 mg/l 2, 4-D to induce somatic embryogenesis in different cultivars. Othmani *et al.* (2009 and 2010) suggested 10 mg/l 2, 4-D for cv. Aslam *et al.* (2011) used 1.5 mg/l 2, 4-D for cvs. Barhee, Zardai, Khalasah, Muzati, Shishi, and Zart, while Khierallah *et al.* (2015) used 50 mg/l picloram for cv. Bream.

Table 2: Effect of 2, 4-D, NAA and BA on periodical response of culture in two local cultivars.

Treatment		**Concentrations of 2,4-D and /NAA along with BA 4.5mg/l**	**Periodical response of tissue**			
			After 70 days	**After 100 days**	**After 130 days**	**After 170 days and above**
ASEEL						
T1	Control	0+0	-	Tissue	Tissue	Dead
T2	NAA +2,4-D	20+15	No response	No response	No response	Callus initiation
T3	NAA +2,4-D	20+25	No response	Callus initiation	Callus formed	Callus regeneration

T4	NAA +2,4-D	20+75	No response	Cell enlargement	Cell enlargement	Callus initiation
T5	NAA +2,4-D	20+100	No response	Cell enlargement	Cell differentiate	Callus initiation
T6	NAA +2,4-D	40+15	No response	Cell enlargement	Callus differentiate	Callus initiation
T7	NAA +2,4-D	40+25	No response	Cell enlargement	Cell enlargement	Callus initiation
T8	NAA +2,4-D	40+75	No response	Cell enlargement	Cell enlargement	Cell enlargement
T9	NAA +2,4-D	40+100	No response	No response	No response	Cell enlargement
T10	NAA +2,4-D	80+15	No response	No response	No response	Cell enlargement
T11	NAA +2,4-D	80+25	No response	No response	No response	Cell enlargement
T12	NAA +2,4-D	80+75	No response	No response	Cell enlargement	Cell enlargement
T13	NAA +2,4-D	80+100	No response	No response	Cell enlargement	Cell enlargement
KARBALAIN						
T1	Control	0+0	-	Tissue	Tissue	Dead
T2	NAA +2,4-D	20+15	No response	No response	No response	Callus initiation
T3	NAA +2,4-D	20+25	No response	Callus initiation	Callus formed	Callus regeneration
T4	NAA +2,4-D	20+75	No response	Cell enlargement	Cell enlargement	Callus initiation
T5	NAA +2,4-D	20+100	No response	Cell enlargement	Cell differentiate	Callus initiation

T6	NAA +2,4-D	40+15	No response	Cell enlargement	Callus differentiate	Callus initiation
T7	NAA +2,4-D	40+25	No response	Cell enlargement	Cell enlargement	Callus initiation
T8	NAA +2,4-D	40+75	No response	Cell enlargement	Cell enlargement	Cell enlargement
T9	NAA +2,4-D	40+100	No response	No response	No response	Cell enlargement
T10	NAA +2,4-D	80+15	No response	No response	No response	Cell enlargement
T11	NAA +2,4-D	80+25	No response	No response	No response	Cell enlargement
T12	NAA +2,4-D	80+75	No response	No response	Cell enlargement	Cell enlargement
T13	NAA +2,4-D	80+100	No response	No response	Cell enlargement	Cell enlargement

Effect of 2, 4-D, NAA and BA on the different quality parameters of callus

Callus nature, color, weight and growth of two different local date palm varieties was observed in

Table. 3, which shows that the callus was white creamish and friable (**Fig.1**), when T3 was applied. Whereas, the higher concentrations of NAA had modular compact in both varieties (Table. 3). The growth and weight was varied with the use of different concentrations. The growth of callus was good depending on the type of cultivar and its weight was higher at low concentrations of auxin. Similar findings were also reported by Tisserat 1982; Eke *et al.* 2005 with respect to quality of callus. Yadav *et al.* (2001) also obtained creamish white and friable callus with use of 100 mg/l 2, 4-D, 3-5 mg/l 2ip and 80 mg/l Adenine later the callus was transferred on the MS basal media without hormones for somatic embryo maturation, germination and shoot proliferation.

Table 3: Effect of 2, 4-D, NAA and BA on the different quality parameters of callus in two different local cultivars.

Treatment		Concentrations of 2,4-D and /NAA along with BA 4.5mg/l	Periodical response of tissue			
			Nature	Colour	Weight (g)	Growth
ASEEL						
T1	Control	0+0	-	-	-	-
T2	NAA +2,4-D	20+15	Friable	Creamish white	0.30	Little
T3	NAA +2,4-D	20+25	Friable	Creamish white	0.60	Good
T4	NAA +2,4-D	20+75	Friable	Creamish white	0.28	Fair
T5	NAA +2,4-D	20+100	Friable	Creamish white	0.25	Little
T6	NAA +2,4-D	40+15	Friable	Creamish white	0.24	Little
T7	NAA +2,4-D	40+25	Friable	Creamish white	0.32	Little
T8	NAA +2,4-D	40+75	Nodular compact	Brownish	0.19	-
T9	NAA +2,4-D	40+100	Nodular compact	Brownish	0.10	-
T10	NAA +2,4-D	80+15	Nodular compact	Brownish	0.15	-
T11	NAA +2,4-D	80+25	Nodular compact	Brownish	0.18	-

T12	NAA +2,4-D	80+75	Nodular compact	Brownish	0.10	-
T13	NAA +2,4-D	80+100	Nodular compact	Brownish	0.20	-
KARBALAIN						
T1	Control	0+0	-	-	-	-
T2	NAA +2,4-D	20+15	Friable	Creamish white	0.32	Little
T3	NAA +2,4-D	20+25	Friable	Creamish white	0.62	Good
T4	NAA +2,4-D	20+75	Friable	Creamish white	0.30	Fair
T5	NAA +2,4-D	20+100	Friable	Creamish white	0.26	Little
T6	NAA +2,4-D	40+15	Friable	Creamish white	0.25	Fair
T7	NAA +2,4-D	40+25	Friable	Creamish white	0.30	Fair
T8	NAA +2,4-D	40+75	Nodular compact	Brownish	0.10	-
T9	NAA +2,4-D	40+100	Nodular compact	Brownish	0.18	-

T10	NAA +2,4-D	80+15	Nodular compact	Brownish	0.15	-
T11	NAA +2,4-D	80+25	Nodular compact	Brownish	0.10	-
T12	NAA +2,4-D	80+75	Nodular compact	Brownish	0.20	-
T13	NAA +2,4-D	80+100	Nodular compact	Brownish	0.10	-

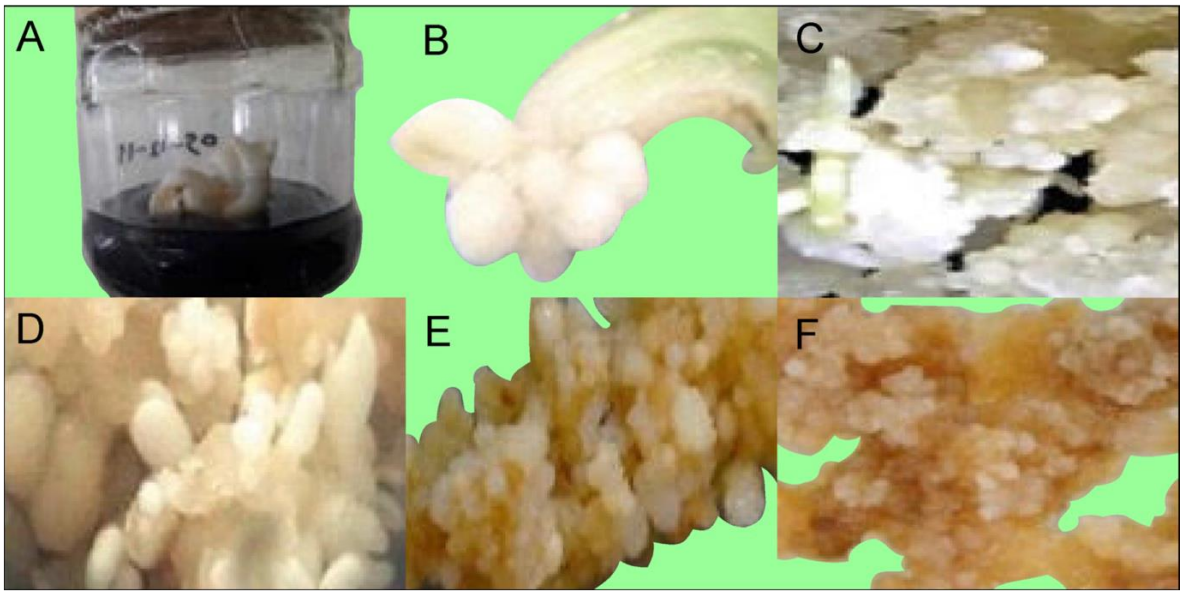

Figure 1: (A) Explant in media (B) Callus Initiation (C) White Creamish Callus (D) Somatic Embryogenesis (E&F) Browning in Callus.

Conclusion:

Multiplication of date palm *in vitro* has proven to be a very efficient procedure to accelerate the production of high quality, disease free healthy plantlets for genetic, physiological uniformities and are fast growing. While traditional method of propagation of offshoots/suckers from mother plant is time consuming practice, those suckers bring diseases and insect pests along with them. To achieve this *in-vitro* generated biotechnological protocol for callus culture and somatic embryogenesis was optimized in present studied. The combination of 2,4-D, NAA

and BA at lower concentrations i-e; 20+25+4.5 mg/l were found efficient for getting good callus and embryogenesis a way forward to develop disease free date palm plantlets.

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