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SALIVA ANTIGEN TEST AS A NEW BIOMARKER IN POPULATION-BASED SCREENING OF CORONAVIRUS INFECTIONS

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MARYAM TAHA YASEEN AL-GAYYIM

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By Maryam Taha Yaseen AL-GAYYIM

January 2022

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

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ABSTRACT

SALIVA ANTIGEN TEST AS A NEW BIOMARKER IN POPULATION-BASED SCREENING OF CORONAVIRUS INFECTIONS

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Master of Science in Chemical Engineering

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Saliva testing is becoming more common. It contains salivary gland secretions, desquamated oral epithelial cells, and microorganisms. There are also immunoglobulins, mucins, enzymes, metabolites, hormones, and electrolytes. This makes saliva ideal for early covid 19 diagnosis. This study examines oral specimens and compares SARS-CoV-19 antigen detection assay results with CRP and other markers. Cross-sectional study of 19 covids. 100 patients with a full history, exam, and covid19 lab tests provided blood and saliva. PCL COVID19 Ag (saliva test) detects SARS-CoV-19 antigen in human saliva. Diagnostic accuracy for SARS-CoV-2 in blood and saliva varied. The CRP test found 79% in group1 and 66% in group2, while the saliva test found 6% and 7%. D-dimer tests found 80% in group1 and 76% in group2. PCL SARS-CoV-2 saliva antigen test accuracy in early/late infection was below manufacturer specifications. Remember this when testing. DIAGNOS may be an easy-to-use saliva complement to detect SARS-CoV-2 positive individuals, but validation with other biomarkers and diagnostic tools using larger sample sets is needed.

2022, 38 pages

Keywords: Saliva antigen test, Biomarker, Coronavirus, Infections

ÖZET

KORONAVİRÜS ENFEKSİYONLARININ NÜFUS TEMELLİ TARAMALARINDA YENİ BİR BİYOMARKER OLARAK TÜKÜRÜK ANTİJEN TESTİ

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Haziran 2022

Tükürük testi daha yaygın hale geliyor. Tükürük bezi salgıları, pul pul dökülmüş oral epitel hücreleri ve mikroorganizmalar içerir. Ayrıca immünoglobulinler, müsinler, enzimler, metabolitler, hormonlar ve elektrolitler de vardır. Bu, tükürüğü erken covid 19 teşhisi için ideal hale getirir. Bu çalışma, oral numuneleri inceler ve SARS-CoV-19 antijen saptama testi sonuçlarını CRP ve diğer belirteçlerle karşılaştırır. 19 covid'in kesitsel çalışması. Tam öykü, muayene ve covid19 laboratuvar testleri olan 100 hastaya kan ve tükürük sağlandı. PCL COVID19 Ag (tükürük testi), insan tükürüğünde SARS-CoV-19 antijenini tespit eder. Kan ve tükürükte SARS-CoV-2 için teşhis doğruluğu değişiyordu. CRP testi grup1'de %79, grup2'de %66, tükürük testi ise %6 ve %7 olarak bulundu. D-dimer testleri grup1'de %80, grup2'de ise %76 olarak bulundu. Erken/geç enfeksiyonda PCL SARS-CoV-2 tükürük antijen testi doğruluğu üretici spesifikasyonlarının altındaydı. Test ederken bunu unutmayın. DIAGNOS, SARS-CoV-2 pozitif bireyleri tespit etmek için kullanımı kolay bir tükürük tamamlayıcısı olabilir, ancak daha büyük numune setleri kullanan diğer biyolojik belirteçler ve teşhis araçları ile doğrulama gereklidir.

2022, 38 sayfa

Anahtar Kelimeler: Tükürük antijen testi, Biyobelirteç, Coronavirüs, Enfeksiyonlar

PREFACE AND ACKNOWLEDGEMENTS

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Maryam Taha Yaseen AL-GAYYIM

Çankırı-2022

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LIST OF SYMBOLS

%	Percent sign
µL	Microliter
g	Gram
mL	Milliliter

LIST OF ABBREVIATIONS

ACE-2	Angiotensin converting enzyme 2
COVID-19	Coronavirus disease-19
CoVs	Coronaviruses
IFN	Interferon
MD	Mild disease
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
RTC	Replication and transcription processes
SARSr-CoV	SARS-related coronavirus
SD	Severe disease
TMPRSS	Transmembrane protease

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1. INTRODUCTION

CoVs are single-stranded RNA viruses that infect birds and mammals and are members of the large subfamily Coronavirinae. The viral RNA genome is the longest one yet discovered, measuring anywhere from 26 to 32 kilobases. Seven CoVs have been identified as pathogens in humans, and they are classified as either lowly or highly pathogenic (Xu 2020). Four HCoVs have been identified as SARS-like coronaviruses (HCoV 229E, NL63, OC43, and HKU1). However, they offer only a tiny risk to human health. SARS-CoV, now known as SARS-CoV-1, the Middle East respiratory syndrome coronavirus (MERS-CoV), and SARS-CoV-2 have all appeared in the last two decades, producing devastating infections in humans each time they are encountered. (Raoult, the year 2020) (Rothan and Byrareddy 2020). Because it is caused by SARS-CoV-2, it is known as Coronavirus Diseases-2019 (COVID-19). While the SARS-related coronavirus (SARSr-CoV) is genetically linked to the original virus, the illness symptoms of the two viruses vary (LOW et al. 2021). Polymerase chain reaction (PCR) confirms the presence of COVID-19, which is initially identified by X-ray or CT scans (Wu *et al.* 2020).

In the Coronaviridae family, the Coronavirinae and Torovirinae subfamilies and the Coronavirinae subfamily's four genera make up SARS-CoV-2. In addition to HCoV-229E and HCoV-NL63 found in the Alphacoronavirus, other coronaviruses include HCoV-OC43, SARS-HCoV, HCoV-HKU1, and the Middle Eastern Respiratory Syndrome coronavirus (MERS-CoV). Gammacoronaviruses, found in whales and birds, are found in the Alphacoronavirus, while viruses found in pigs and birds are (FEHR *et al.* 2015).

SARS-CoV-2 has a common ancestor with SARS and MERS-CoV, two extremely deadly viruses, since it is a Betacoronavirus. Single-stranded RNA pathogen SARS-CoV-2 has a negative sense of direction, as suggested by its name (Kramer *et al.* 2006).

ACE-2 receptors are used by both SARS-CoV and SARS-CoV2 to infect airway epithelial and endothelial cells, and the genomes of both viruses are 75 percent similar (Li 2019).

2. LITERATURE REVIEW

2.1 Structure of Coronavirus 2

Coronaviruses are well known for their club-shaped spikes on the surface of their virion. The crown-like form of the Coronavirus is what gives it its name (Fehr 2015). As is usual for Coronaviruses, the genome of CoV-2 lacks the segmented positive-sense, 5-capped, 3-poly A tail structure (Ashour 2020). All four of the most significant coronavirus proteins are encoded by the coronavirus genome: S, N, M, and E. (Figure 2.1). For cell membrane fusion, the S protein has two S1 and two S2 regions, which are responsible for binding receptors (Lu 2019).

On the virus's outer coat is a transmembrane protein. By attaching to the lower respiratory tract cell-expressed ACE2 protein, the S protein assists in the attachment of envelope viruses to host cells. S. Glycoprotein S1 and S2 are broken down by the host cell's furin-like protease. A virus's host range and cellular tropism are determined by part S1 of the receptor binding domain make-up, whereas part S2 facilitates viral fusion in transmitting host cells using S2 (Guo 2020).

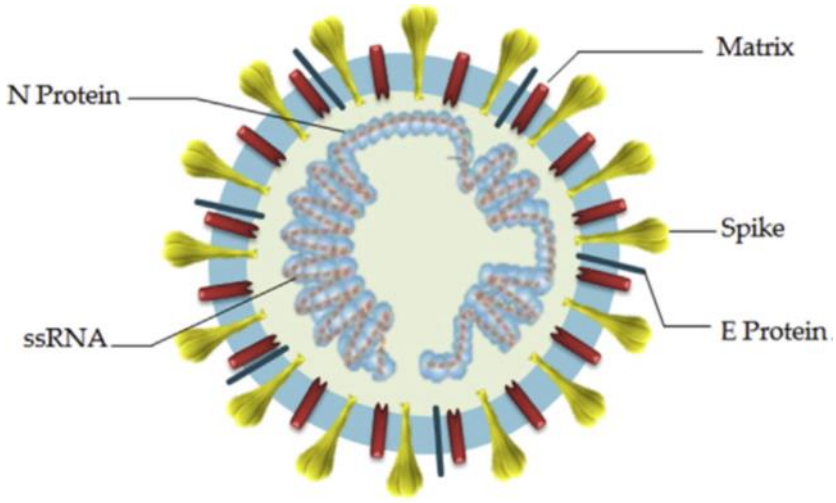

Figure 2.1 Structure of coronavirus2 (Tai 2020).

The coronavirus N protein is structurally connected to the virus's nucleic acid in the endoplasmic reticulum-Golgi region. Being attached to RNA, the protein is essential to the viral genome's replication and the immune response of infected cells to viral infection. Because of this, there's a reason why this is happening Because RNA is connected to the protein. Phosphorylation may produce structural changes in N protein, boosting viral RNA's ability to interact with protein (Fehr 2020). Virus envelope shape is heavily influenced by the most structurally complex protein in the virus, the M protein. All of the structural proteins can be bind to this protein, making it one of a kind. Virion assembly is facilitated by M protein binding to nucleocapsids (N proteins) and N protein-RNA stabilization (Schoeman 2021). weighing in at 8.4–12 kD, the E protein, which contains 76–109 amino acids, is a tiny and integral membrane protein (Raamsman *et al.* 2000). This affects virus production and maturation. This protein, which is involved in several stages of the viral life cycle, including budding, envelope formation, and pathogenesis, is a viable target for inhibiting pathogenesis (Pervushin, 2020).

2.1.1 Life cycle of coronaviruses

For example, SARS virus binding to the type II pneumocyte ACE-2-receptor causes the lower respiratory tract to become inflamed after the virus has infiltrated the respiratory tract (Kuba *et al.* 2020). Type 2 transmembrane protease (TMPRSS) has been shown to cause ACE-2 proteolytic cleavage when SARS spike protein binds to the ACE-2 receptor, resulting in spike protein activation (Heurich *et al.* 2014). SARS-CoV is more likely to enter cells that have both of these proteins present at the same time (Shulla *et al.* 2020). For the SARS-CoV2 virus to enter the body, it must infect all cells (Zhou *et al.* 2020).

Another way for SARS-CoV-2 to enter is via its internal S2′ subunit (Figure 2.2). While the S1–S2 border polybasic sequence is cleaved in an infected cell, the S2′ site is cleaved in the target cell following ACE2 engagement. An opening in the S2′ cleavage site is created when the SARS-CoV-2 virus binds to ACE2, causing the S1 subunit to undergo conformational alterations. Five additional steps are required. When the virus

enters the host, various proteases break the S2′ site of SARS-CoV-2. This stage involves clathrin-mediated endocytosis (clathrin-mediated endocytosis) and cathepsins, which need an acidic environment to function, cleaving the virus–ACE2 complex in end lysosomes (step 3). (3) and (4). The S2′ cleavage of S2 in the presence of TMPRSS2 can be accomplished in two different ways (JACKSON 2022).

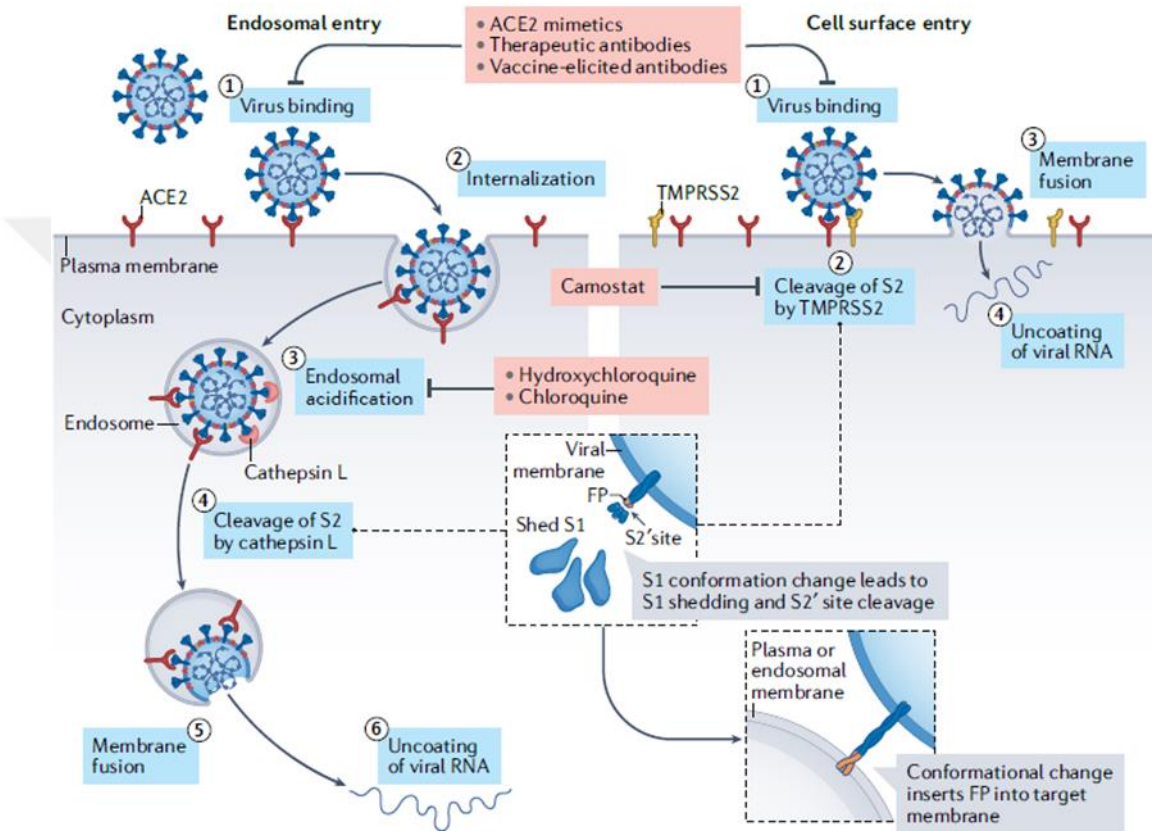

Figure 2.2 Two SARS-CoV-2 pathways (JACKSON 2022)

Heptad repeat 1 of S2 subunit dissociates from the S1 subunit and propels FP into the target membrane, triggering both entrance routes (step 5 on the left and step 3 on the right). RNA from viruses enters the host cell via an opening generated when viral and cellular membranes fuse to release into the cell's cytoplasm for uncoating and replication (step 6 on the left and step 4 on the right) (JACKSON 2022).

Immature RNA is carried out of the nucleus by a single stranded RNA particle. RTC is the acronym for "replication/transcription complex," which is responsible for both

replication and transcription (RTC). The viral genome contains non-structural proteins that make up this complex. Double-membrane cytoplasm formations have been attributed to the RTC's influence on the infected cell (Van Hemert 2021). As of (Van Hemert, 2021). Proteins that replicate in the cell are made from open reading frames after a successful translation of the RNA genome. Generated full-length negative sense RNA is utilized to make more full-length genomes, which are then employed in the process again and again. The cytoplasmic synthesis of M, S, and E is transported to the endoplasmic reticulum-Golgi intermediate compartment (ERGIC) for further processing (Song 2020). This song is set to be released in 2019. Encapsulation of replicated DNA genomes by N protein results in the formation of nucleocapsids, which combine with the ERGIC membrane to create new virions in the cytoplasm. Newly generated virions are released from infected cells and transferred to the cell membrane in smooth-walled vesicles through exocytosis. Overworking the endoplasmic reticulum during viral generation results in cell death (Masters 2006).

2.1.2 Pathogenesis and immune response

The body's innate immune system serves as the initial line of defense against viral infections for most people (IIS). Infected cells produce and secrete interferon (IFN) molecules in response to virus infection. They act as signaling molecules for the immune system, activating an antiviral response in the cells around them and making them resistant to infection. Macrophages produce and IFNs quickly and efficiently after a viral infection has occurred, only a few hours later (Huang 2019). Interferon-inducing type I interferons (IFN-I/II) are known to be suppressed by SARS coronavirus (SARS-CoV). Adaptive immunity is activated by the 4th to 7th day after an infection if the body's first line of defense, the innate immune system, fails to eliminate pathogens (Cologne 2021).

As the illness evolves from a moderate to a severe stage, COVID-19 has varied amounts of numerous cytokines and chemokines (TUFAN *et al.* 2020). A specific adaptive immune response needs to be initiated early in the COVID 19 incubation and nonsevere stages in order to eliminate the virus and stop it progressing to a severe stage. Antiviral

cytotoxic T cells can stop viral replication by neutralizing free virus particles (CTLs). IFN alpha/beta and IFN-gamma are two examples of innate cytokines that play a role in regulating and initiating adaptive immune responses (Thevarajan 2020). (Thevarajan 2020). NK, CD4, and CD8 Th1 cells, as well as cytotoxic suppressors and interferon-alpha/beta-producing infected cells, are among the immune system cells that do not produce IFN- (Fan 2020).

CTLs and NK cells, two types of cytotoxic lymphocytes, are critical in the fight against viral infection. Cytotoxic lymphocyte functional exhaustion is linked to disease progression (Zhang 2019). Compared to patients with moderate disease (MD), patients with severe disease (SD) had considerably higher neutrophil counts, although lymphocyte counts were significantly lower. Patients with MD and SD have considerably less CD8+ T cells and T cells. T cell and CD8+ T cell numbers were considerably lower in SD patients compared to MD individuals The NK cell counts of SD patients were much lower than those of MD patients (Li 2013).

Blood IL-2, IL-7, IL-17, IL-10, MCP-1, MIP-1A and TNF- are all greater in ICU patients compared to those who are not in the ICU. There have been studies that show that in patients with both severe and non-severe infections, plasma concentrations of IL-2, IL-6, IL-8, IL-10, and TNF- are much greater in the latter (Qin 2019). This cytokine, known to contribute to macrophage activation syndrome (MAS), is elevated in both the mild and severe COVID-19 patient groups, with severe patients having a higher plasma level than mild patients (Chen 2019).

One of the most pressing problems to be addressed is the evaluation of biomarkers in COVID-19 hospitalized patient populations. Reverse transcription PCR, serology, symptoms, and a chest MRI were all used to confirm COVID-19 infection in each study. There were numerous biomarkers to take into account.

2.2 Detection tools for COVID-19 infection

Polymerase Chain Reaction in reverse transcription (RT-PCR)

Small quantities of viral genetic material were amplified together with other nucleic acid sequences using the gold standard technique of RT-PCR for upper respiratory tract samples to identify SARS-COV-2.

All patients with a strong suspicion of infection should undergo urgent RT-PCR testing for SARS-COV-2, according to the WHO. The presence of SARS-COV-2 has been verified by RT-PCR. A clinical suspicion of infection may need further testing if the results are negative (e.g., epidemiologic link or typical x-ray findings). In the event that the second test comes back negative, a serological investigation is considered (WHO 2020). It's possible that the RT-PCR findings are incorrect if there aren't enough oropharyngeal swab samples available (Li *et al.* 2020). False negative RT-PCR findings in individuals with COVID-19 pneumonia may delay identification and early treatment, increasing the risk of transmission to the general population (Li *et al.* 2020). Chest CT scans are increasingly being requested by patients who think they may have pneumonia and want to be diagnosed and treated as soon as possible (Li *et al.* 2020).

2.3 Iron and Ferritin

Iron is found in cells in two different forms: Fe (II) and Fe (II) (III). All oxidation–reduction reactions require Fe (II)-containing proteins as cofactors and catalysts because of their ability to transfer electrons and high solubility. In contrast, the stable Fe (III) form of iron is used for storage and transportation. Extra iron atoms in the bloodstream result in the production of superoxide anion and the hydroxyl radical, which can damage cells by depleting oxygen and hydrogen peroxide of electrons. For example, the Fenton reaction can be used to generate ROS by oxidizing organic compounds with Fe (II) and H2O2 (Pignatello 2006).

Iron is an essential nutrient for the body, but it also has the potential to be toxic. Oxygen transport, cellular respiration, and the formation of free radicals are all dependent on the presence of this enzyme. All iron-using organisms bind iron atoms to proteins to prevent this kind of damage. All organisms have ferritin as their primary intracellular iron storage protein (VLAHAKOS 2021). It has been suggested that a number of different indicators of iron status, including hemoglobin and cell volume (MCV), as well as serum iron and total iron binding capacity (TIBC), may be used to determine a person's iron status (Northrop 2008).

In 2019, the various organ dysfunction condition produced by Coronavirus illness is exacerbated by the iron metabolism. (COVID-19). A mechanism that might cause anemia during infections is the innate immune response's restriction of iron availability in order to deprive the pathogen of it (Ganz 2019). To put it another way, anemia may have a considerable impact on multiple organ failure (TANERI 2020).

All organisms have ferritin as their primary intracellular iron storage protein. Thousands of Fe3+ Atoms Can Be Adsorbed by Ferritin. Up to 1 g of iron can be stored in the human body, most is bound to ferritin. MW 440 kDa ferritin forms a hollow ball composed of a dozen 19-21 kDa polypeptide subunits that can contain up to 3000-4500 ferric atoms. Ferritin is a polypeptide that is composed of two dozen subunits. The subunits can be classified as H or L based on their mass. The iron-loading of ferritin is dependent on the ferroxidase activity of the H-subunit. In ferritin nucleation and stabilization, the L-subunit is thought to play a role. Small amounts of ferritin (50-200 g/dL) are found in human plasma, proportional to the total body stores of ferritin. Iron stores in the body can be estimated by monitoring plasma ferritin levels (H).

2.4 C-reactive Protein

Inflammation biomarker C-reactive protein (CRP) is a protein generated by the liver in response to interleukin-6 (IL-6) (Acad 2004). An elevated CRP level may simply be an indicator of inflammation in the body. Because of the production of IL-1, the IL-6, and

TNF, the liver produces CRP as a consequence of an inflammatory stimulation (YEH 2004).

A wide variety of infectious and noninfectious disease severity and treatment response have been linked to chronic granulomatous inflammation (CRP) (Clyne *et al.* 1999). A variety of respiratory disorders, such as severe acute respiratory syndrome, Middle East respiratory syndrome, and H1N1 influenza, have been related to elevated levels of C-reactive protein (Ko 2016). Recent research suggests that increased CRP levels in individuals with COVID-19 may be associated to the disease's severity and progression (Herold 2020). There is a lot of potential in the CRP biomarker for prognosis.

2.5 D.Dimer

When active Factor XIII, calcium, and platelets are present, thrombin cleaves fibrinogen into fibrin monomers that may be aligned and cross-linked through covalent bonds. When plasmin dissolves cross-linked fibrin, it creates D-dimers and other fibrin degradation byproducts. The D-dimer may be assessed as a diagnostic test to rule out thromboembolic illnesses or to maintain track of DIC. D-dimer may be utilized as a marker for both hemostasis and fibrinolysis since it is created during fibrinolysis (Giannitsis and colleagues 2017)

After the index thrombus is formed, it has a half-life of approximately 8 hours and can be detected in blood approximately 2 hours later (Giannitsis *et al.* 2017). As a result, increased levels of this parameter suggest that COVID-19-related severe thrombosis and even pulmonary embolism may occur due to increased fibrinolytic activity (Ulanowska *et al.* 2021) D-dimer levels below 0.5 mg/L were found in 46.4% of patients in China who had non-severe disease, but only 43.4% had elevated D-dimer levels, and about 60 percent of those patients had severe illness (Guan *et al.* 2020). This was done in accordance with ISTH guidelines for measuring D-dimer in conjunction with platelet count, PT, and fibrinogen in COVID-19 patients (Thachil 2020)

2.6 Saliva Test

It has been suggested that salivary testing might be used to identify the coronavirus that causes severe acute respiratory syndrome (SARS). Proteins and digestive enzymes guard against infection in salivary excretion (Dawes 2019). Saliva contains more than just gingival crevicular fluid and serum; it also contains expectorated airway surface liquid and mucus, epithelial and immunological cells from the upper airways, and oral bacteria and viruses (Miller 2010). It is commonly utilized as a diagnostic tool for both oral and systemic disorders despite the fluid's mixed sources (Dawes 2019). Dengue, West Nile, Chikungunya, Ebola, Zika, and Yellow Fever have all been related to coronaviruses SARS and MERS (Niedrig 2018).

Researchers claim that the use of 100-l Saliva samples avoids the problems associated with invasive, high-quality Nasopharyngeal swabs by providing a more reliable, less variable alternative. In the morning, after people have just brushed their teeth or used mouthwash or another oral hygiene product, high-quality samples can be distributed. Saliva self-harvesting has a number of advantages, including reducing the risk of infection to those conducting the sampling. At least 20 days without viral transport medium at 4°C is required for sample stability (ii). (iii) Less time and money are spent on testing because RNA extraction is not required. Increased throughput and lower costs are possible with sample pooling (iv). V) Not competing with other diagnostic schemes, allowing manufacturers to increase capacity without putting additional strain on them. Reduced supply chain issues, as demonstrated by the worldwide shortage of nasopharyngeal swabs during the outbreak of Ebola (Moreno-Contreras 2021). With a high level of accuracy comparable to that of the traditional method of testing for influenza, this method has been found to be effective. (vii) (Teo 2021).

2.7 The Knowledge Gap and Importance of the Study

Patients with symptoms and individuals undergoing repeated screening to determine the validity of saliva tests as an alternative sample source for RT-PCR testing require proof that saliva can be used as an alternative sample material for RT-PCR testing.

A recent study suggested that saliva could be used instead of swabs for the diagnosis of Covid-19 (Sabino-Silva *et al.* 2020), but the advantages of saliva and the scientific evidence that supports the idea that saliva analysis in patients with Covid-19 may allow for a more accurate diagnosis are discussed herein. Biomarkers for this disease could be discovered using saliva, as well as guidelines for better use of this sample (To *et al.* 2019).

Because of the difficulty of tracking COVID-19 infections through repeated sampling (Khurshid *et al.* 2020). Saliva is a non-invasive, self-collected specimen that can be used for public health surveillance. For the new generations of covids 19, most research on saliva use has taken place among in-patients and symptoms-affected ambulatory patients, with little focus on a community-based screening setting. The advantages of saliva over other covid 19 testing methods include the following:

Patients can collect their own nosocomial infections at home, so there are no health care workers who are exposed to them. It's easier to control crowds in clinical settings because there is less need for medical personnel and less time spent waiting for samples to be collected (Tvarijonaviciute *et al.* 2020)

It's easy for patients to accept because it's not painful or stressful. Large-scale or epidemiological studies, as well as serial sampling in certain populations, such as children, can all benefit from using this technique (Azzi *et al.* 2020)

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Study design

This study analyzed (100) patient samples in a cross-sectional fashion. Imam Hussein Medical City/Kerbala and Imam Sadiq Hospital/Babylon collected COVID-19 patient samples. In addition, they had a medical scan to look for signs and symptoms of COVID-19. They were also subjected to a medical examination by a specialist doctor looking for signs and symptoms of COVID-19 according to WHO criteria.

The questionnaire used in this study was derived from a review of relevant literature and discussion among the researchers. Participants' responses were guaranteed to be kept confidential and private in order to minimize the bias that could be introduced by self-reported data.

3.1.2 Materials Instruments

As can be seen in Table 3.1, every equipment and tool utilized in this investigation was listed.

Table 3.1 The research instruments that were utilized

Instrument	Suppliers
Centrifuge	HETTICH/ Germany
Deep Freeze	COOLTECH/ China
ELISA system	UNO/HUMAN/ Germany
Roller Mixer	China

3.1.3 Tools, materials and Kits

A list of the tools, materials, and kits utilized in this research and the firms who provided them is provided in Table 3.2 and Table 3.3.

Table 3.2 Materials and equipment used in the research

Pipette(100-1000µl)	DRAGON MED/ USA
Micropipette(10-100 µl)	DRAGON LAB/ USA
Gilson Tips,1000µl (blue)	China
Gilson Micro-tips, 100µl	China
Eppendroff Tubes	China
Gel tubes	China
Sodium citrate tubes	China
EDTA tubes	China
Gloves	China
Syringe	China

Table 3.3 The study's tools and supplies

Chemicals	Source
kit of PCL COVID19 Ag (saliva lest)	Republic of Korea PCL, Inc.
Kit of D-dimer	Snibe Maglumi 800/Germany
Kit of C-Reactive protein	China

3.1.4 Criteria for inclusion and exclusion

Criteria for inclusion: "All patients with full signs and symptoms of COVID-19 who were in the hospital for one day up to two weeks must be reported as confirmed cases of COVID-19." They were going through a full medical history, a clinical scan, and relevant laboratory tests.

3.1.5 Exclusion criteria

It had no exclusion criteria, so all confirmed cases of COVID-19 were included.

3.1.6 The Ethical committee's approval

It was accepted by the college's ethics committee and the Al-Hayat unit of Imam Hussein Medical City's Al-Hayat unit. Patients' or loved ones' consent was required for any sample collection.

3.1.7 Measurement and data collection

Patients' age, gender, and current chronic illness status were collected using the self-reported approach (student questionnaire) (Hypertension, Diabetes, and Autoimmune diseases). During the exams, data on the smoking habits of the patients, their body temperatures, their dry coughs, their oxygen saturation levels, and their breathing difficulties were collected.

3.1.8 Blood and saliva collection and storage

In order to draw 5 ml of blood, a disposable syringe containing 5 ml of blood was inserted into the patient's vein. Three sections of blood were separated:

1) Two milliliters of blood were placed in a gel tube and allowed to sit at room temperature for fifteen minutes before analysis. The serum was separated by centrifugation at 4000 rpmxg for 10 minutes. A serum sample was collected using an eppendroff and stored at -20°C in order to avoid multiple freezing and thawing cycles. Blood samples were used to determine CRP levels.

2) A sodium citrate anticoagulant tube was used to collect three ml of blood. Ten minutes of centrifugation at approximately 4000 xg resulted in the separation of plasma. D-dimer concentrations were measured in plasma.

We used disposable, non-pyrogenic, and endotoxin-free tubes to collect the blood.

3.1.9 Saliva specimen collection

30 seconds of saliva collection from the tip of the tongue in the mouth of the test subject is required (approximately 0.5 mL). To expedite extraction, the collected saliva was spit directly into the extraction buffer tube. The tube's volume increased by about half after the saliva was added.

3.2 Methods

3.2.1 C-Reactive protein as a marker of inflammation

Method: Method of Turbidimetry

Principle: Equation 3.1 showed a correlation between the concentration of CRP and an increase in absorbency when measuring the immunocomplex formed by CRP antibodies and CRP in the sample Table 3.4.

$$Anti - human\ CRP\ antibody\ +\ CRP\ \leftrightarrow\ Immunocomplex\ (agglutination) \qquad (3.1)$$

Preparation of reagents:

Table 3.4 The CRP Kit's components and concentrations

R1:	Tris buffer PEG Surfactant	100 mmol/L 0.26 mmol/L <2% (m/v)
R2:	Tris buffer anti-human CRP Antibody (goat)	100 mmol/L dependent on titer

Calculation: Equation 3.2 was automatically generated after calibration by the analyzer for each sample.

$$Conversion\ factor:\ \frac{mg}{dL}x\ 0.1 = \frac{mg}{dL}\ , Or: C\ sample = \frac{\Delta A\ sample}{A\ calibration}\ xC\ calibration \quad (3.2)$$

3.2.2 Tests for the D-dimer

Principle: Immunoassay using D-Dimer sandwich chemiluminescence. The material was treated with a magnetic microbead sandwich containing a monoclonal antibody directed against D-Dimer. The supernatant was then poured over the microbeads and a wash cycle was carried out. " In the flashchemiluminescent process, starters 1 and 2 were introduced. It was quantified in terms of relative light units (RLUs), which were directly linked to the concentration of D-Dimer in samples, using a photomultiplier Table 3.5.

Table 3.5 Parts of a D-dimer kit

Components	Contents	100 tests (REF:13060600 8M)	50 tests (REF:13 0606008 M)
Magnetic microbeads	Monoclonal antibody-coated magnetic microbeads containing 0.1 percent BSA and NaN3 (anti-Dimer monoclonal antibody).	2.5Ml	2 mL
Calibrator Low	Containing less than 0.1 percent NaN3, D-Dimer antigen is BSA-based.	2.5mL	2 mL
Calibrator High	BSA and NaN3-containing D-Dimer antigen.	2.5mL	2 mL
Buffer	Bovine serum albumin (BSA) and NaN3 (less than 1%).	6.5 mL	4 mL
ABEI Label	An ABEI-labeled monoclonal antibody, which is comprised of BSA and NaN3, is available (less than 0.01 percent).	6.5 mL	4 mL
Internal Quality control	Containing less than 0.1 percent NaN3, D-Dimer antigen is BSA-based.	2 mL	2 mL
* We have everything you need to get started.			

Reagent preparation: To ensure that magnetic microbeads were properly rehydrated prior to use, the kit was loaded with magnetic microbeads that were automatically resuspended.

Alleviation: The reagent kit did not include instructions on how to dilute samples for analysis. Manual dilution was required for samples with concentrations above the detection limit. To get the final result, the dilution factor was multiplied.

Calculation of results: The D-dimer level in each sample was automatically calculated by the analyzer using a 2-point calibration master curve approach.

3.2.3 Method of saliva antigen test in population-based screening of corona virus infections

Principles of procedure: Nucleocapsid (N) protein of SARS-CoV-2 is detected by PCL COVID19 Ag Gold. Gold-labeled COVID19 antibodies are attached to nitrocellulose mem-brane near the test card sample hole, where the antibodies can be detected (as shown in the figure below). Capillary forces draw the sample from the sample hole to the test area of the device after its application. COVID19 antibodies separate from the membrane and move along the test card as sample liquid passes over them Figure 3.1.

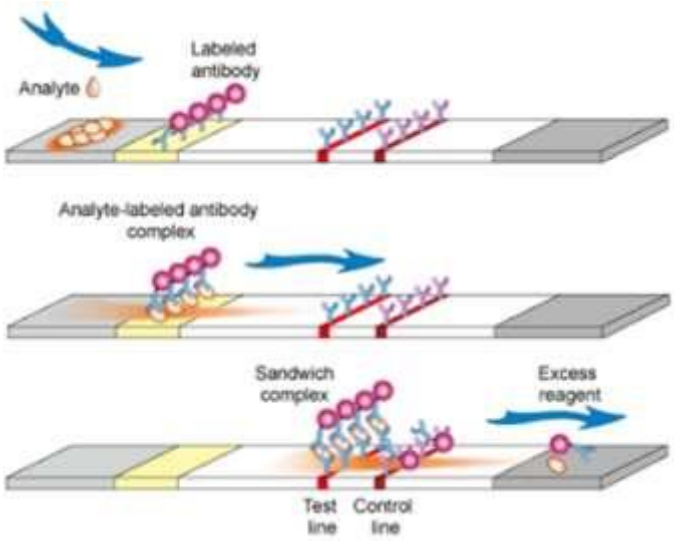

Figure 3.1 Principles of the procedure of Saliva Antigen Test

Tagged antibodies bind to analyte-labeled antibody complexes, which are formed when the sample contains SARS-CoV 2 antigens (the "analyte"). A second set of COVID19

anti-bodies, which are immobilized on the nitrocellulose membrane, retains these complexes on the test line when they reach it. The test line appears to have a color band around these so-called sandwich complexes. Sandwich complexes are not formed if the sample does not contain antigens from SARS-CoV-2.

The control line of the test card will show a color band regardless of whether or not the sample contains SARS-CoV-2 antigens. This means that the test card did not work as intended if there is no color band on the control line.

Kit preparation: Before testing, reagents were given a 20-30 minute rest at room temperature Figure 3.2.

Assay procedure for saliva specimens:

1. the samples were Collected and used directly.

2. Filter caps were placed on the tubes, and the lid was then tightened. When the tube was turned upside down ten times, the contents were agitated.

3. It was only after the pouch had been opened that the test cards were used.

4. Flat surfaces were used to place the test card. It was necessary to add a few drops of the saliva extraction buffer to the test card's sample hole before mixing it in completely.

5. After a ten-minute wait, the results were revealed.

6. Results were Interpretated based of the following figure

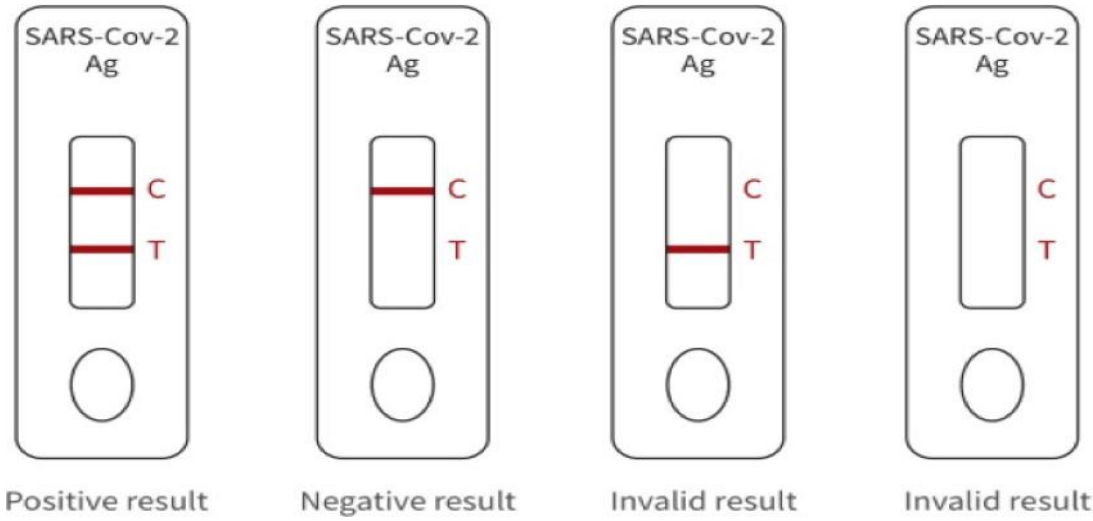

Figure 3.2 Interpretation results of Saliva Antigen Test in Population-Based Screening of Corona virus infections

3.3 Statistical Analysis

Input into a spreadsheet of patients' responses to a questionnaire and the results of tests was performed. Multiple entries were used as a precautionary measure. According to these findings, SPSS version 28.0 was employed to conduct this research (IBM, SPSS, Chicago, Illinois, USA) Real Statistics Resource Pack for Mac may also be used to do statistical analysis with Excel 2016. (Release 7.2). (2013-2020).

Each group's participants' data was analyzed using descriptive statistics. Analyses of both continuous and categorical variables were conducted using the mean and standard deviation (SDs). Box plots and Shapiro tests were used to establish the data's normality.

These considerable differences between the parameters were verified using statistical analysis. All hypothesis tests were judged statistically significant if the p-value was less than 0.05 (two-sided).

4. RESULTS AND DISCUSSION

4.1 Demographic and Clinical Characteristics

Table summarizes the COVID-19 groups' clinical and laboratory demographics and results Table 4.1. The age range of participants was shown in the table, which was between the ages of 12 and 95. The male-to-female ratio in the study groups was 52:48 for the patients' group. People with moderate to severe fevers, shortness of breath and bad cough were the most commonly reported symptoms.

COVID-19 infection rates are higher in men than in women, according to several studies. Social and biological factors are the primary causes of these sex differences (Gebhard *et al.*, 2020). Men are more likely to smoke and lead unhealthy lives than women when it comes to the most common COVID-19-related comorbidities.

Males are also more likely to engage in cultural norms that put them at greater risk of illness, infection spread, or underutilization of medical services (Gebhard *et al.* 2020). Males are more susceptible because they have a higher concentration of ACE 2 receptors than women (Hoffmann 2020).

Only four COVID-infected patients in group 1 were confirmed by positive PCR and saliva tests. Only ten patients in group 2 had a positive PCR, according to the data. The relative merits of PCR methods in terms of approach, cost, labor, deployment route, and result turnaround have been reported. However, the use of nasopharyngeal swabs is a common problem (Torjesen 2021). Even when performed by highly skilled medical professionals, reaching the correct nasopharynx target site is a common error. If you're not an expert, or if you're doing it yourself, you have a 50 percent chance of swabbing in the wrong place and for too long. There may be some discomfort as the swab becomes lodged in the middle turbinates (Higgins 2020), which discourages a regular testing schedule. False negatives encourage people to relax and engage in

risky behaviors, which in turn increases viral transmission. There is a distinct difference between COVID-19 symptoms and COVID-19 testing results.

Table 4.1 Demographics of the participants in the study

Characteristics		Grouping of patients based on the length of time they've had the infection		Normal range
		Group 1 (≤ 5 days) (n= 62)	Group 2 (>5 days) (n= 38)	
Demographics	Mean Age	56.67	64.35	/
	Gender (n) (Male/Female)	(29 , 34)	(23, 14)	
PCR results (n)	Positive	4	10	/
	Negative	58	28	
CRP (n)	Positive	49	25	0-6 mg/L
	Negative	13	13	
Saliva test (n)	Positive	4	3	/
	Negative	58	35	
Biochemical parameters Mean±2SD	D-dimer Mean±2SD	1949.54± 749.53	1596.19± 729.45	<500 ng/mL

4.2 Analysis of the Distribution of Information Among the Groups Studied

Using a box plot to illustrate the distribution of the data, the quartiles (or percentiles) and averages were depicted. Using box plots, you can see a data set's five most important numbers: the lowest score, the median, the third-highest quartile, and the maximum score. Line that divides box shows median, which is the average value from a given dataset. This statistical term refers to how far an observed distribution has been stretched or compressed (also known as variability, scatter, or spread). At the very ends of the 'whiskers,' we find the smallest and largest values, which are useful for showing the range of measurements. Data showed that patient group interquartile ranges had greater dispersion and more variability, but the figures also showed this.

4.3 Study the Distribution of Biomarkers Levels in COVID Patients

In addition, the researchers studied D-dimer levels, which might be utilized to differentiate between individuals in the moderate and severe stages of the illness (Fu *et al.* 2020) Both groups had D-dimer concentrations of 1782 ng/mL. Patients who had been diagnosed with COVID-19 for more than five days exhibited substantially high levels of D-dimer and CRP in their serum or other body fluids, as shown in Figure 4.1.

84 percent of COVID19 patients had elevated CRP levels, which is consistent with other studies (Chen 2019). Patients who had been infected for a longer period of time had a much higher CRP level than those who had been infected for a shorter period of time (Tan 2020).

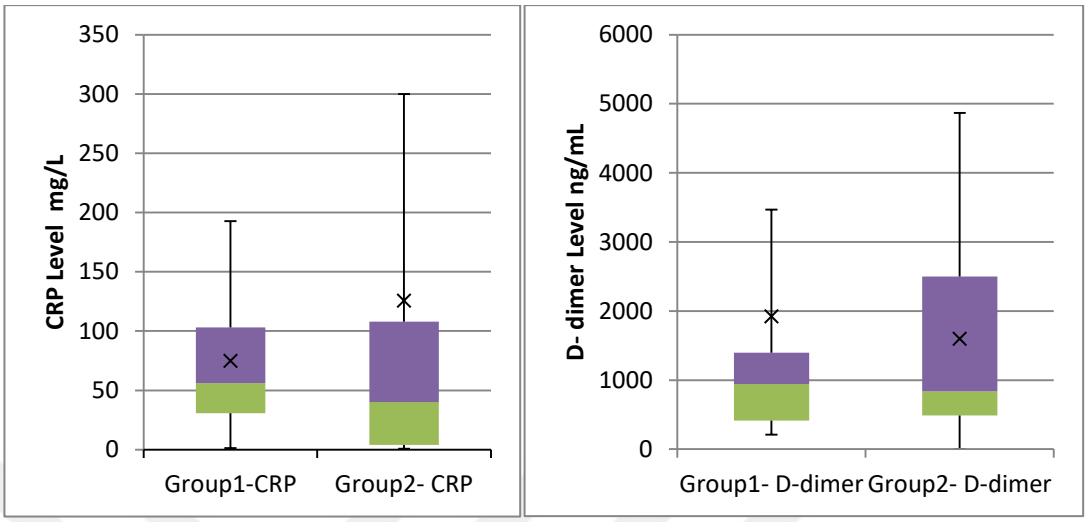

Figure 4.1 A boxplot of the inflammatory indicator's blood level distribution (CRP) and Coagulation parameters(D-dimer) in early diagnosed COVID-19 patients Group 1 (≤ 5 days) and Later diagnosed COVID-19 patients Group 2 (>5 days)

4.4 Correlation Between Levels of Inflammatory Indicator of COVID-19 (CRP) in Case of Positive/ Negative PCR and Saliva Antigen Test

According to the PCR results, the distribution of CRP in various stages of COVID-19 patient groups is shown in Figure 4.2. CRP levels varied depending on the severity of the illness. CRP levels averaged 63.1 mg/L in the early diagnostic group (group 1) with a negative PCR and 76.5 mg/L in group 2.

Table 4.2 shows that the mean PCR concentrations were 50.6 mg/L in group 1 and 130.9 mg/L in group 2 in those groups that had positive results. After a patient was PCR positive, CRP levels spiked, indicating that the COVID was being overworked. Increasing the average levels of PCR had only a weak correlation with the outcomes studied.

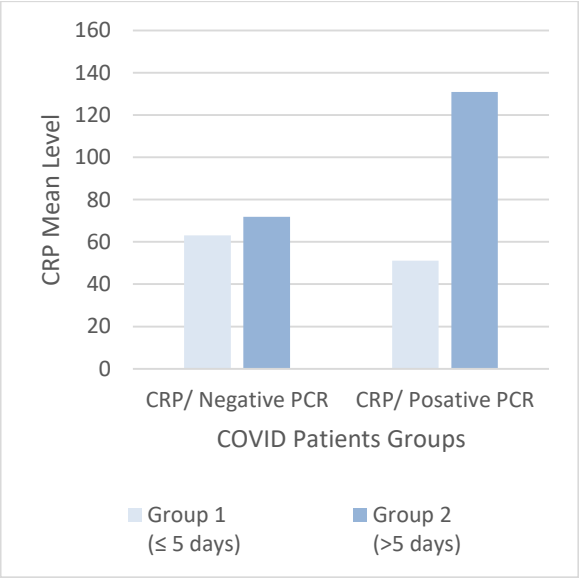

Figure 4.2 In early COVID-19 patients Group 1 (5 days) and later COVID-19 patients Group 2 (>5 days) with Negative and Positive PCR test results, the mean CRP level was altered

Table 4.2 CRP baseline characteristics and Spearman rank test correlation coefficients in COVID-19 patients with (A) Negative and (B) Positive PCR tests.

(A)		Negative PCR	
Patients Groups	Mean	Spearman Correlation Coefficients (rs)	p-value
CRP G1	63.1	-0.4	0.1
CRP G2	76.5		
(B)		Positive PCR	
Patients Groups	Mean	Spearman Correlation Coefficients (rs)	p-value
CRP G1	50.6	-0.42	0.5
CRP G2	130.9		

Inflammatory markers such as CRP may be more suitable for universal screening. The liver secretes CRP in response to injury or infection, which raises blood levels. The complement and mononuclear phagocytic systems are activated by CRP, resulting in viral clearance. Predictors of disease severity can be correlated with changes in CRP levels during acute inflammation and infection.

On the other hand, Levels of CRP was increased among COVID-19 patients groups. In both COVID-19 patient groups CRP which confirmed plus Positive Saliva antigen test as shown in Figure 4.3. High prognosis values were found compared to Negative Saliva group.

CRP levels were shown a weak relationship with the duration of infection , p values were >0.05 as illustrated in Table 4.3.

There are statistically significant changes in inflammatory markers and immune cells as a result of infection. Since COVID-19 is associated with both viral and bacterial infections, CRP levels can be used to better predict the disease's progression (Zhang *et al.* 2019)

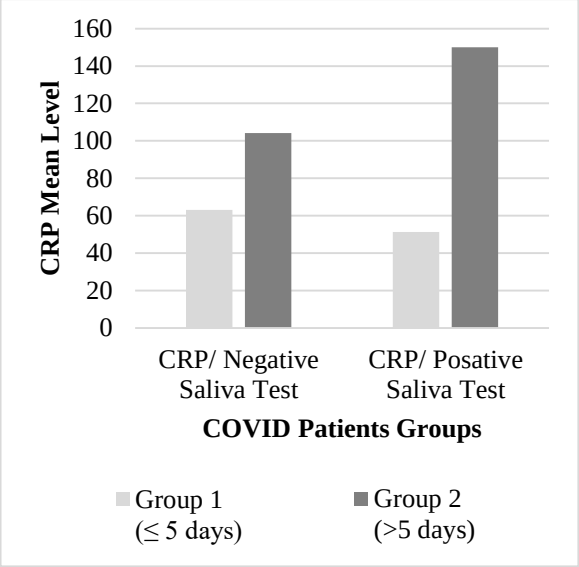

Figure 4.3 An increase or decrease in the mean C-reactive protein level in COVID-19 individuals who were diagnosed early (within 5 days) compared to those who were diagnosed later (within 5 days or more) Analyze of antigens

Table 4.3 Baseline characteristics and Spearman rank test correlation coefficients between CRP in COVID-19 patients with (A) Negative and (B) Positive Saliva antigen tests.

	Positive Saliva			
	Patients Groups	Mean	Spearman Correlation Coefficients (rs)	p-value
	CRP G1	50.7	0.42	0.14
	CRP G2	130.9		

	(A) Negative Saliva			
	Patients Groups	Mean	Spearman Correlation Coefficients (rs)	p-value
	CRP G1	65.5	0.36	0.085
	CRP G2	104.2		

4.5 Correlation Between Levels of Coagulation Parameters (D-dimer) of COVID-19 in Case of Positive/ Negative PCR and Saliva Antigen Test

It may be possible to distinguish patients in the early stages of the illness from those in the later stages by comparing their D-dimer levels (Fu *et al.*, 2020). PCR-positive patients in Groups 1 and 2 had considerably higher levels of serum D-dimer, as shown in Figure 4.4. With the help of Spearman's rank correlation, we discovered a strong correlation (0.82) between the differences between two groups and a statistically significant P value (0.04) Table 4.4. The correlation between D-dimer and patients with negative PCR results P 0.05 was non-significant, but the correlation was weak.

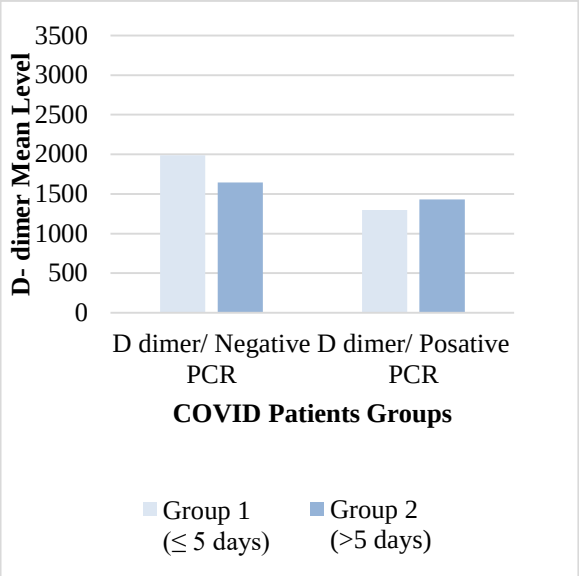

Figure 4.4 Covid-19 patients with Negative and Positive PCR results in the early and later stages of the disease, as well as those with D dimer levels of more than 5 days old

Table 4.4 D dimer baseline characteristics and Spearman rank test correlation coefficients in COVID-19 patients with (A) Negative and (B) Positive PCR tests

(A) Positive PCR			
Patients Groups	Mean	Spearman Correlation Coefficients (rs)	p-value
Ddimer G1	1241.7	0.82	0.04
Ddimer G2	1429.2		
(B) Negative PCR			
Patients Groups	Mean	Spearman Correlation Coefficients (rs)	p-value
Ddimer G1	1989.9	-0.4	0.2
Ddimer G2	1655.8		

On the other hand, the correlation between saliva Antigen Test results and D-dimer levels was examined. In Figure 4.5 the mean levels of D-dimer in group 2- with positive saliva Antigen were increased more than third time compared to patients who were having Negative Saliva test. The Correlation Coefficient was associated weakly with non-significant p value (> 0.05) as shown in Table 4.5. While in case of Negative

Saliva test, group two indicated decreased levels of D-dimer compared to group 1, non-significant differences were found (p value > 0.05) as shown in Table 4.5.

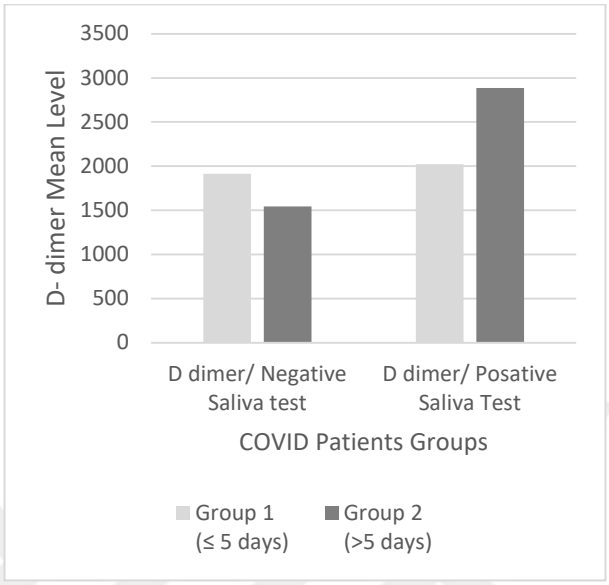

Figure 4.5 In both a positive and negative sense Early COVID-19 patient Group 1 (5 days) and later COVID-19 patient Group 2 (>5 days) D dimer saliva antigen testing

Table 4.5 COVID-19 patients with positive or negative saliva antigen test results reveal a substantial association between D dimer levels and saliva antigen test findings

(A) Positive Saliva			
Patients Groups	Mean	Spearman Correlation Coefficients (rs)	p-value
Ddimer G1	2007.6	0.44	0.12
Ddimer G2	2885.8		
(B) Negative Saliva			
Patients Groups	Mean	Spearman Correlation Coefficients (rs)	p-value
Ddimer G1	1913.34	-0.38	0.068
Ddimer G2	1544.6		

During the course of the disease, dynamic changes in D-dimer levels have been shown to be a predictor of poor outcomes (Li 2020). When the coagulation cascade begins, the Fibrinolytic system kicks in and works to keep the clot in check. Fibrinolysis is an

enzyme-based method for separating blood clots into D-dimers. For example, pulmonary embolism and deep vein thrombosis may be diagnosed with the fibrinolysis indicator D-dimer (Lee *et al.* 2020). D-dimer levels have also been linked to pneumonia (Yao *et al.* 2020).

D-dimer levels in COVID-19 may be elevated due to clogged blood arteries and inadequate oxygenation. As a result, D-dimer levels tend to grow in COVID-19 patients at critical stage, with a high link (Zhou *et al.* 2020)

5. CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

In this study, the researchers found that CRP, D-dimer, and saliva antigen tests were associated with poor outcomes in COVID-19 patients. As a biomarker for the treatment of high-risk COVID-19 patients, the findings could lead to better prognoses and lower mortality rates.

Furthermore, the PCL SARS-CoV-2 saliva antigen test's diagnostic accuracy was significantly lower than the manufacturer's specifications in an early/late infection period. The following should be taken into account when developing testing plans. A larger sample set and validation with other biomarkers and diagnostic tools are needed to confirm that the DIAGNOS is an effective and simple saliva complement for the detection of SARS-CoV-2 positive individuals.

5.2 Recommendations

COVID-19 diagnostic tools are an active research area, and more data are needed to clarify their similarities and differences. The potential use of a marker that could indicate the progression of infection and endothelial dysfunction or tissue injury through a proposed mechanism is especially relevant in this regard. Saliva testing as an early marker for COVID-19 or its descendants needs further investigation.

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