Coronavirus(SARS-CoV-2) Pandemic for Dental Practitioners

Subjects: Microbiology

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In the context of the SARS-CoV-2 (Severe acute respiratory syndrome coronavirus 2) pandemic, the medical system has been subjected to many changes. Face-to-face treatments have been suspended for a period of time. After the lockdown, dentists have to be aware of the modalities to protect themselves and their patients in order not to get infected. Dental practitioners are potentially exposed to a high degree of contamination with SARS-CoV-2 while performing dental procedures that produce aerosols. It should also be noted that the airways, namely the oral cavity and nostrils, are the access pathways for SARS-CoV-2. In order to protect themselves and their patients, they have to use full personal protective equipment. Relevant data regarding this pandemic are under evaluation and are still under test.

SARS-CoV-2 COVID-19 pandemic in dental practice COVID-19 risk assessment in dentistry Coronavirus Disease ACE2 receptor Flügge's droplets MAS superior standard hepa filter

1. Introduction

In December 2019, an outbreak of pneumonia appeared in Wuhan City. Wuhan is an important international trading centre in central China. This pathology was concluded to be generated by a novel Coronavirus (nCoV-2019). Since then, the virus infection has spread throughout the world, it has been declared a pandemic by WHO on 12 March 2020 [1][2][3]. It seems that the first COVID-19 (coronavirus disease 2019) cases were connected to a large fish and living animal market in this large metropolis. It was thought that the path of direct transmission came from a food market. Since then, person-to-person transmission has been found be one of the main spreading mechanisms of COVID-19 [1][2][3].

After the identification of the initial cases, the pandemic hit almost all the nations in the world. Now, there are more than 1,113,307 deaths worldwide due to the coronavirus pandemic. The updated data of Johns Hopkins University identified 1,113,307 deaths. On the other hand, 39,964,414 contagions are global. COVID-19 has spread to 189 countries and territories and there are approximately 39,964,414 confirmed cases (as of 19 October 2020) [4].

The WHO (World Health Organization) presented the guidance for case management of COVID-19 in health facility and community Interim on 19 March 2020 [3]. The response interventions proposed by the WHO are presented in Figure 1.

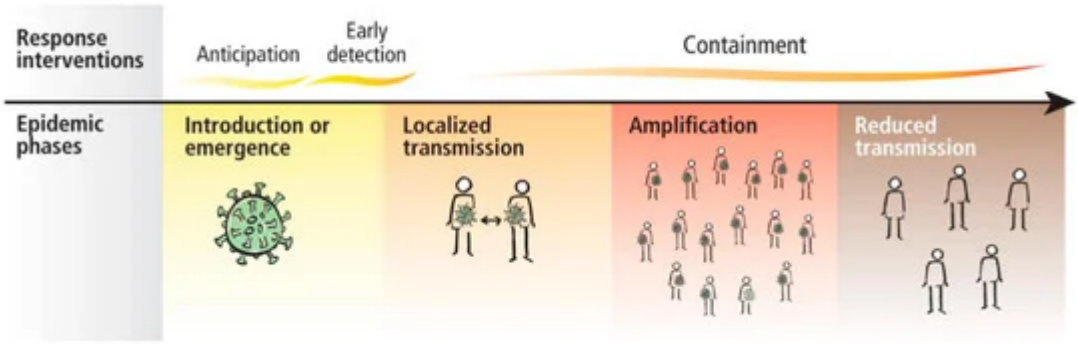

Figure 1. Operational considerations for case management of COVID-19 in health facility and community-Interim guidance 19 March 2020 [3].

Because this pandemic emerged in our lives and has produced a lot of changes, dental professionals have to introduce new strategies to perform dental treatments in order to reduce the risk of cross infection. A study performed by a team of Jordanian dentists showed that dental practitioners have very little information regarding the measures they have to take in order to protect themselves and their patients [5]. In his study, Ing showed that 4% of deaths were dentists because of the lack of protection equipment [6].

In this article, we made a synthesis about the way in which SARS-CoV-2 spreads, how to diagnose a novel corona virus infection, what the possible treatments are, and which protective personal equipment we can use to stop its spreading.

2. Epidemiology

The first name given to this virus was 2019-nCoV, after a short period of time the name of the virus was changed due to the similarity with the SARS virus into SARS-CoV-2 [7]. The virus comes from the family of Coronaviridae and is made of single stranded RNA viruses [7]. This virus can be secluded from animal species and can determine cross infection, passing the barriers of certain species and infecting animals and humans. The virus has a cover that is composed of glycoproteins that look similar to a solar crown, as shown in Figure 2 [7].

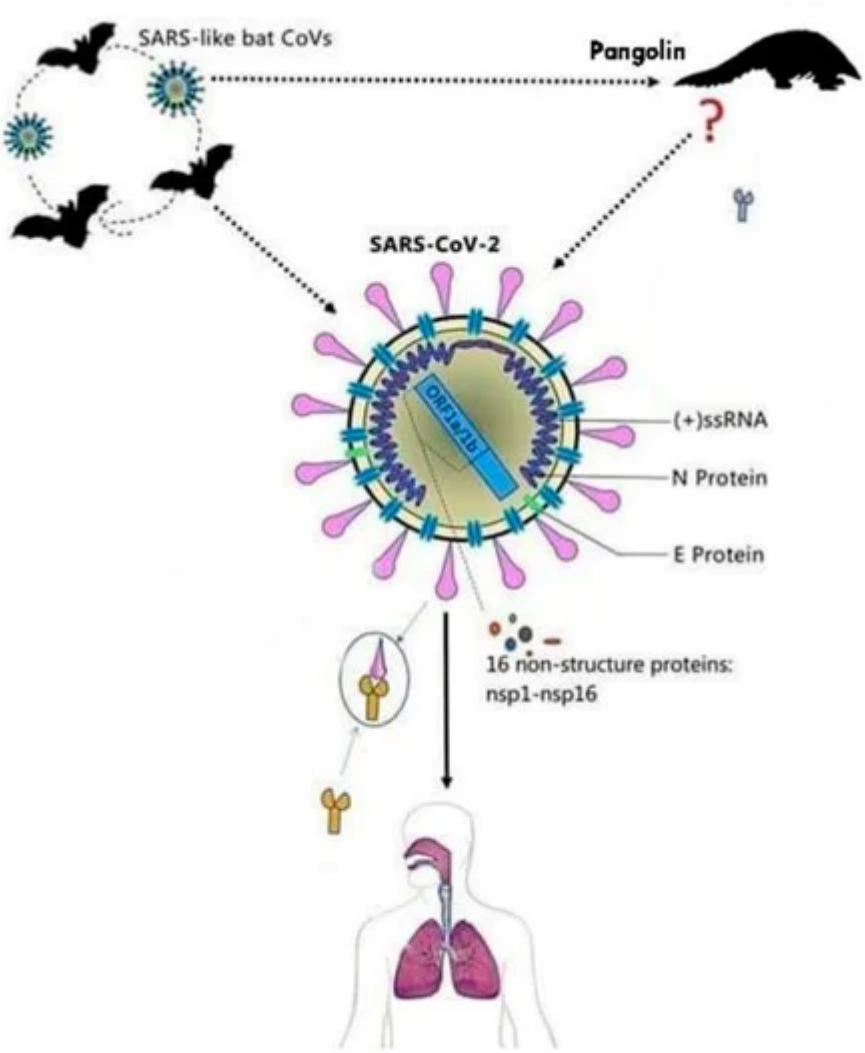

Figure 2. Conformation of SARS-CoV-2 (figure drawn by Giovanna Dipalma).

In the literature, there are four genera of Coronaviruses. Two of the genera, γ-CoV and δ-CoV, determine changes in birds, while the other two genera, α-CoV and β-CoV, contaminate mostly mammals and also humans, by determining changes in different systems of the organism like the respiratory, gastrointestinal, and central nervous systems [7][8][9][10][11]. The new virus that determined infections in Wuhan belongs to the β-CoV family of viruses that includes the SARS-CoV (Severe Acute respiratory syndrome coronavirus) and MERS-CoV (Middle East respiratory syndrome), two viruses that are known for the infections they caused several years ago [8][9][10][11][12][13][14].

The nucleotide sequence similarity between SARS-CoV-2 and SARS-CoV is of about 80% and approximately 50% between SARS-CoV-2 and MERS-CoV. This could explain the reason why this novel virus is less deadly than the other two. Hence, its routes of transmission can spread the SARS-CoV-2 faster than the other two viruses [15][16][17][18][19][20]. It has been suggested that the natural host of SARS-CoV-2 may be the Rhinolophus affine bat, due to the similarities in the RNA (ribonucleic acid) sequence of the coronavirus found in the bat (96.2%) and SARS-CoV-

2, and the intermediate host is the pangolin, with a genome sequence similarity of 99% between SARS-CoV-2 and the coronavirus found in these species [15][16][17][18][19][20].

As far as the intermediate guest is concerned, there are recent studies that contradict the hypothesis of appearance from the pangolin [21]. Some studies say that the first SARS-CoV-2 found in Wuhan's HU-1 patient, in early December 2019, was perfectly adapted to humans, that is, despite being replicated several times in the following months, it did not undergo genomic transcription changes, remaining practically almost unchanged [17][18][19][20][21][22][23][24].

The bat is now considered to be the first source of the virus and the initial hypothesis of the infection from the pangolin that has been considered to be an intermediate host has been discarded [17]. Tang et al. suggested that the genomic sequence between the pangolin virus and the human one is not 99%, but lower (84%) than that the bat one (96%) [22].

Scientists are now taking into consideration the fact that the virus evolved and is infecting humans that were asymptomatic for three months, and in the same manner it increases the infectious capacity and reduces lethality [21].

This type of virus has the same structure as the common coronavirus, possessing the "spike protein" in the exterior structure of the envelope of the virion, and besides this, in its structure we can find proteins like nucleo, poly, and membrane proteins that include RNA polymerase, papain-like protease, helicase, 3-chymotrypsin-like protease, accessory proteins, and glycoprotein. The S protein from coronavirus can bind with the receptors of the host to encourage viral entry into target cells. Although there are four amino acid variations of S protein between SARS-CoV-2 and SARSCoV, the first can also bind with the human angiotensin converting enzyme 2 (ACE2), the same host receptor for the SARSCoV. SARS-CoV-2 cannot bind with cells without the presence of ACE2. The recombinant ACE2-Ig antibody, SARSCoV-specific human monoclonal antibody, and the serum from a convalescent SARS-CoV-infected patient can neutralize SARS-CoV-2, and thus confirms ACE2 as the host receptor for SARS-CoV-2. Due to the high affinity between ACE2 and SARS-CoV-2 S protein, it has been suggested that the population with a higher expression of ACE2 might be more susceptible to Coronavirus Disease 2019 (COVID-19) [17][18][25][26][27].

There is a great number of articles stating the fact that SARS-CoV-2 uses the S protein complex and the ACE2 receptor to entry the host cell. This information is used in different ways and domains, and above all in therapeutic treatment modality, diagnostic purposes, and infection transmission and prevention [28][29][30][31][32][33][34][35][36][37][38][39][40][41][42][43][44][45].

Zhou et al. indicated that the angiotensin-converting enzyme II (ACE2) is likely the cell receptor of SARS-CoV-2, which is also the receptor for SARS-CoV. Moreover, it has been proven that SARS-CoV-2 does not use other coronavirus receptors such as aminopeptidase N and dipeptidyl peptidase 4 [44]. Xu et al. showed that the S-protein of the SARS-CoV-2 supports a strong interaction with human ACE2. Those findings suggest that the ACE2

plays an important role in cellular entry of the SARS-CoV-2, thus, ACE2-expressing cells may act as target cells for susceptible to SARS-CoV-2 infection. Moreover, this research has shown that the ACE2 could be expressed in the oral cavity, which was highly enriched in the oral epithelial cells, especially at a higher level in the tongue than in the buccal and gingival tissues. These findings indicate that the oral mucosa can express a potential high-risk route of SARS-CoV-2 infection transmission [45]. This fact underlines the importance of dentists and dental healthcare workers to wear all the protective measures that are indicated to prevent infection [18].

The connection between the host and the virus is encouraged by the S glycoprotein that integrates the receptors of the host cells to produce the viral infection of the cells. This glycoprotein is part of the class 1 viral fusion proteins and it has more than 1300 amino acids [46].

The SARS CoV 2 can also bind a specific enzyme in the human body that is represented by the angiotensin-converting enzyme 2 (ACE 2), in order to bind the virus to the cells which need this enzyme [47]. The way the entire process takes place is presented in Figure 3.

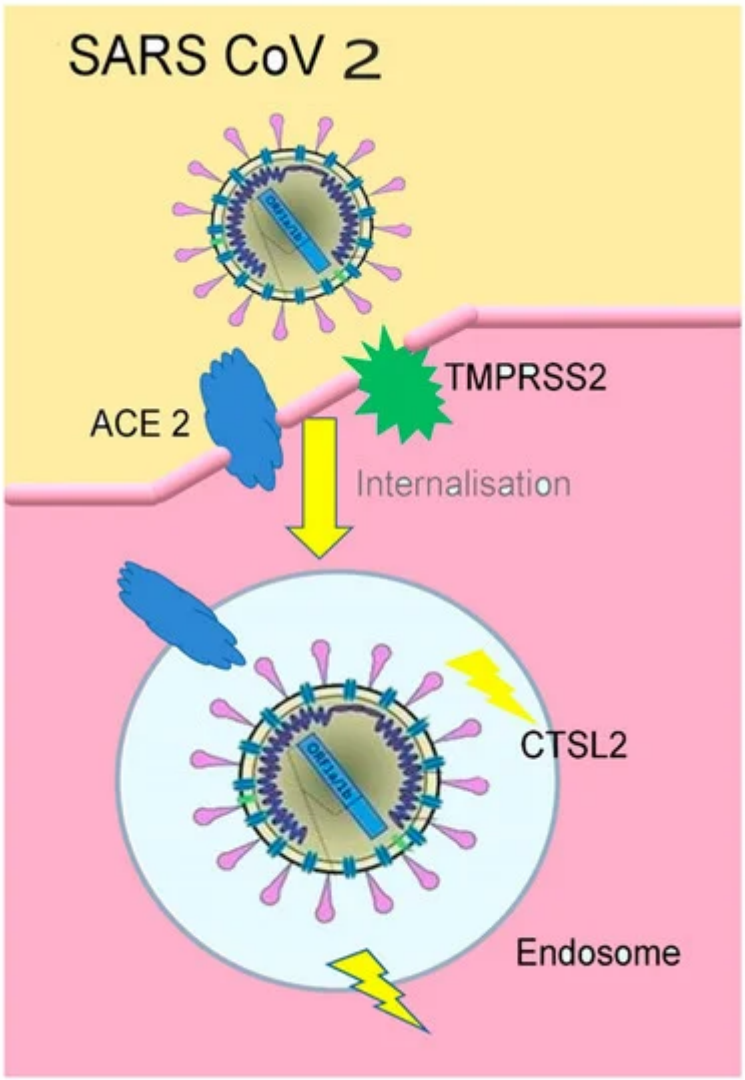

Figure 3. The connection of SARS-CoV2 to ACE2(figure inspired by https://www.the-scientist.com/news-opinion/receptors-for-sars-cov-2-present-in-wide-variety-of-human-cells-67496 [48], and drawn by Giovanna Dipalma).

TMPRSS2(transmembrane protease serine 2) is an enzyme used by the SARS-CoV2 for S protein priming. CTSL2 (Cathepsin L2) is a gene, proteins encoded in this gene are members of the peptidase C1 family. For the SARS-CoV2 virus entering the human cells, Spike (S) protein needs to be cleaved by the cellular enzyme furin [49][50].

Furin is an enzyme, encoded by the FURIN gene, in the cells, belonging to hydrolases, splits proteins (inactive precursors) and transforms them into an active biological state (mature proteins) [44][45].

The S protein allows the virus to transfer the genome into the cell which leads to viral replication. In order to become active, the S protein must be cleaved by proteases. The S protein has two functional domains S1 and S2. S1 is implicated in the initial stage of viral entry, using its receptor binding domain to link to the receptors of the target cell and S2 acts in the second stage, fusing the cell and the viral membrane containing amino acid sequences necessary to continue the infiltration process [50][51][52][53][54].

In the corona virus family, the S protein has the largest variable amino acid sequence. In this virus the furin site is located between S1 and S2 subunits not unlike the pattern found in high-pathogenic influenza viruses, but not in other members of the Beta coronavirus genus [50][51][52].

Several patches of the RBD (receptor binding domain) are similar in SARS-CoV and SARS-CoV-2, an overall amino acid sequence identity of 76.47%. Because of the five important interface amino acid residues, four of these are different in SARS-CoV-2, and its S protein has a significantly higher binding affinity to human ACE2 than SARS-CoV S protein. Regardless of this, the two viruses shared an almost identical 3-D structure of the RBD which demonstrates similar Van der Waals electrostatic properties [50][51][52][53][54].

As treatment options, the effective antibodies against SARS-CoV's spike S protein did not bind to the SARS-CoV-2 S protein. So, a vaccine sounds more tempting, but in order to create a live attenuated vaccine we must limit the replicative capabilities of the virus. In order to achieve this, we could remove the furin activation sequence which is essential for cell–virus fusion, thus allowing it to replicate. By doing so, the immune system can create antibodies in order to neutralise the virus and protect the body from further infections [50][51][52][53][54][55].

Another option would be to isolate antibodies from patients who have recovered from COVID-19 by using the novel S protein structure and mapping its structure in order to mass produce them [50].

In 2012 a new corona virus MERS-CoV (Middle East Respiratory Syndrome Coronavirus) was discovered resulting in a disease that manifests itself as severe respiratory disease with renal failure. The fatality rate was up to 38%. The place of emergencies was the Middle East, specifically countries where dromedary camels were identified as species harbouring the virus. Another outbreak was seen in 2015 in South Korea where the final tally was 36

people killed out of 186 confirmed cases. The SARS-CoV outbreak in southeast China had a worldwide tally of 774 killed from 8096 infected with a fatality rate of 9.6% [56].

3. Viral Load-Inflammation

The world virus comes from Latin and means “poison”. Viruses are small microorganisms whose size varies from 0.2–0.3 µm to 1 µm. They need a host cell for living and reproducing (bacterial, vegetal, or animal). They have a very simple structure with an external cover of glycol proteins and lipids, called an envelope or pericapsid, in which there is a protective coat called capsid surrounding the virus genome. In the literature, there are DNA and RNA viruses, double stranded (DNA virus or dsRNA virus) or single (ssDNA virus or ssRNA) stranded. For the latter there is the “polarity” (consisting in process coding the virus) which can be positive or negative, namely ssDNA, ssRNA-, ssDNA+, or ssRNA+ (coronavirus) [57][58]. The cell replication (cytoplasmic or nuclear) is guided by the genome. The genome of the virus enters the host cell, and in few hours the formation of thousands of viral particles is performed, and they spread in the external environment. The replication of the RNA virus occurs easily with errors, as there is no RNA polymerase during the transcription. The high number of viruses as well as the error high frequency during the transcription are the main factors explaining the fast capacity to evolve proper of the SARS-CoV-2. Resistance to therapy is justified by the RNA mutation, even if it is very small, and allows the virus to avoid the attack of the immune system, continuing to change in terms of response in order to adapt to the constant changes of the genome [57][58].

Corona viruses are classified according to their own nature, structure, genome, and replication. The main feature of viruses is the infection of a special type of cell on which surface there are receptors which are similar to the binding. When binding with the host cell membrane is performed through those receptors, the virus penetrates the cell with its own genome, DNA or RNA. In this way. replication and multiplication of the virus starts. After the virus replication, the host cell usually dies. freeing new microorganisms in the surrounding environment where they can keep on infecting a new host cell having completed their lifecycle [59][60].

The Furin is considerably present in the lung tissue, in the intestine and the liver, this would make those organs as potential target of the 2019-nCoV infection. In dental field, Furin expression has been also revealed by the epithelium of the human tongue and in significant quantities in the squamous cell carcinoma. Researchers have shown a high availability of ACE2 receptors as well as the presence of Furin. Therefore, the tongue has a high risk of coronavirus infection and the SCC increases the risk in case of coronavirus exposure. The cleavage site on the spike similar to Furin plays an important role in spreading the 2019-nCoV virus [61][62][63][64][65][66][67].

At the moment there are some researches in which this site is eliminated, by observing effects or blocking the action of Furin, as issued on Nature [16]. This explains the strategic possibilities which we can sum up in this way:

1. Molecules inhibiting Spike-ACE2.

2. Anti-Serin protease molecules.

3. Molecule inhibiting the HR1 domain (sub. S2).

4. Inhibitors of viral enzymes, namely antivirals [16][68].

The activation of TMPRSS2 (Trans Membrane Protease, Serine 2) is fundamental as SARS-CoV-2 infects the lung cells, SARS-CoV-2 can use the TMPRSS2 to trigger the S protein. Some studies highlight that the TMPRSS2 is an important element of the host cell as it is essential for spreading a great number of viruses causing potentially significant infections, as the influenza A and coronavirus. Important data show that the TMPRSS2 is not necessary for the development and homeostasis and so it is potentially and sensible pharmacological target able to inactivate the infection. It is important to underline that the serine protease inhibitor, camostat mesylate, blocks the TMPRSS2 activity. This treatment, or something similar with likely increased antiviral activity (Yamamoto et al., 2016), could be used for treating patients with SARS-CoV-2 infection. Further studies suggest that the activation mediated by Furin on the S1 / S2 site within the infected cells could activate the subsequent access depending on the TMPRSS2 within the target cells [69][70][71][72][73].

An analysis on the real proteolytic elaboration of the protease on the S protein, and on its cleavage in S1 and S2 through detection with the antigenic system, underlined the existence of a band corresponding to the subunit S2 and protein S of the host cells infected by the virus of the vesicular stomatitis (VSV) containing SARS-2-S [61][62][63][64][65][66][67].

Knowing the action of the SARS-CoV-2 may allow to produce targeted drugs and vaccines against the COVID-19, a new treatment modality investigated is the one using PRP (platelet rich plasma), PRF (platelet rich fibrin), and CGF (concentrated growth factors) [74][75][76][77][78][79][80].

In 2011, a study on macaques infected by coronavirus with severe lung infections has been taken into consideration as the saliva droplets were source of infection [81].

It has been confirmed that the epithelial cells of the salivary glands covering the salivary ducts had high ACE2 expression (Angiotensin-Converting Enzyme 2), and therefore the first target cells have been revealed together with the first production source of virus [46][49][50][82][83][84][85].

The ACE2 expression in human organs has been analyzed by considering data collected by the portal Genotype-Tissue Expression. It is noted that the ACE2 expression in minor salivary glands was higher than the ones found in lungs. As a result, salivary glands are targets for SARS-CoV [82].

Another confirmation derives from the fact that the SARS-CoV-2 may be recorded in the saliva before lungs lesions appear. This explains the presence of asymptomatic infections. Therefore, it is possible to state that the salivary gland is not only the first access site for the SARS-CoV-2, but also one of the main reproduction sources, as it makes saliva highly infective and infecting [81][82][83]. Indeed, the high presence of corona virus SARS-CoV-2 in saliva of COVID19 patients reaches 91.7%, and from their saliva samples it is also possible to easily cultivate the virus in vivo [83][84][85][86][87][88][89].

There is a study analyzing the virus SARS-CoV-2 resistance to the internal surfaces and to the sun light. This study proved that the UV-C light (absent to the natural light) inactivates coronaviruses and that the UVB levels found in sun light may really inactivate the SARS-CoV-2 on surfaces, especially the dry virus on stainless steel specimens. This research provided the first evidence that sun light may quickly inactivate the SARS-CoV-2 on surfaces. Data suggest that the natural sun light may be also effective as a disinfectant for contaminated non-porous materials [90]. Researchers have also revealed that the simulated sun light is quickly able to inactivate the corona virus SARS-CoV-2 on specimens performed on stainless steel. The results of this study highlighted that 90% of the infecting virus was inactivated in a period of time consisting of 6.8 min in the saliva solution. The sun light necessary for those tests is similar to the summer solstice, in a not cloudy day. Researchers stated that the inactivation has been tested when the sun light levels were also lower [90][91].

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