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## Characteristic Abnormalities in Saliva Biochemistry in Patients with COVID-19: A Systematic Review and Meta-Analysis

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### Abstract

Background: Outbreak pneumonia announced in Wuhan, China, in December 2019, had its causative factor classified as a new coronavirus (SARS-CoV-2). Since saliva can host several viruses including SARS-CoV-2, the transmission chance of viruses through saliva, particularly those causing respiratory infections, is unavoidable. COVID-19 can be detected through salivary diagnostic testing which has lots of advantages for medical care professionals and patients. However, the utility of saliva in diagnosing COVID-19 infection remains understudied. Aim of the study: In this review, we attempt to provide a comprehensive and updated source of information about salivary markers of liver enzyme, cytokines and oxidative stress in associated with the infections caused by COVID-19 and the various effects it has had on the dental profession and patients visits to dental clinics. Methods: A systematic search was conducted in PubMed/Medline, EMBASE, and Google Scholar, Scopus, and Web of Science, and Google Scholar from December 1, 2020, till April 15, 2021. Results: Nine studies were included in this study. Pooled results showed a significant increase in salivary liver enzymes (ALT, AST, ALP, GGT among patients with Covid-19. Three studies showed a significant elevations in salivary TNF- $\alpha$ , CRP, IL-6 and,IL-10 levels among patients with Covid-19. Four studies that reported a significant in elevation salivary level of MDA, whereas reduction salivary levels of SOD, catalase, GPX. Conclusion: Our meta-analysis shows that deranged of salivary liver enzymes, hyper-inflammation, may indicate severe COVID-19 and could also predict mortality. Larger studies are needed to evaluate the relationship between derangement in salivary biochemical and mortality in COVID-19.

## Introduction

### Coronavirus disease 2019 (COVID-19)

In December 2019, a SARS-CoV-like coronavirus, the 2019 novel coronavirus (2019-nCoV), has emerged in Hubei Province of China and has spread rapidly in mainland China and to other parts of the world. The 2019-nCoV belongs to Betacoronavirus genus lineage B, and is phylogenetically closely related to bat SARS-like coronaviruses. However, the spike, ORF8, and ORF3b proteins differ significantly from other known SARS-like coronaviruses, which may confer differences in pathogenicity and transmissibility from SARS-CoV. Similar to SARS-CoV, the 2019-nCoV can be efficiently transmitted between humans. Cases of familial clustering have been reported. Although a well-regulated innate immune process is the first protection action against viral infections, in severe COVID-19 condition occurs hyper inflammation (“cytokine storm”) that might lead to the acute respiratory distress syndrome (ARDS) (1-6).

The clinical symptoms of COVID-19 are cough, fever, shortness of breath, muscle pain, sore throat, confusion, chest pain, headache, rhinorrhea(4%), diarrhea, and nausea and vomiting. SARS-CoV-2 transmits human-to-human by either direct transmission such as cough, sneeze, and droplet inhalation, or contact transmission like ocular contact, saliva, mucous membranes of the nose and eyes (7).

Rapid and accurate diagnostic tests are essential for controlling the SARS-CoV-2 pandemic. Nevertheless, biological specimen collection is an important logistic challenge to provide massive testing. The possibility to use self-collected samples for COVID-19 testing offers several advantages, especially to minimize the risk of exposing health-care workers to the virus, since self-collection does not require direct involvement of trained personnel in the sample collection. Studies have demonstrated that several important hematological and biochemical markers are altered in patients with COVID-19(8-10). These biomarkers may be important for prognostication and

management of patients, particularly those with co-morbidities and/or a severe course of the disease.

### Saliva and oral health

The oral cavity is a major portal of entry for pathogens that may lead to changes in the normal microflora. The homeostasis of the oral cavity is maintained by saliva (11). Saliva is mainly secreted by the parotid gland, the submandibular gland and the sublingual gland. The submandibular gland serves an role in basal salivary secretion, while the parotid gland serves an important role in stimulated salivary secretion. However, the sublingual gland serves an minor role in salivary secretion.

Saliva is secreted 90% from major salivary glands and 10% from minor salivary glands within pH from 6 to 7. It is an exocrine secretion consisting of approximately (99.5–99.8%).of water In a healthy state, humans produce between (500ml-1.5L) of saliva per day, and contains a variety of electrolytes and proteins, and therefore, as slightly acidic pH, similar to that of plasma. Like other body fluids, saliva is a dilute aqueous fluid, with an osmolality less than or equal to that of plasma, and so, it promotes oral health by constantly bathing the teeth and oral mucosa and thereby serving as a cleaning solution, a lubricant, a buffer and an ion reservoir of calcium and phosphate. Like the blood, saliva reflects body's health status by changes in levels of its constituent proteins, hormones, antibodies and other molecules that are otherwise, measured in standard blood tests, to monitor health and disease(12).Salivary secretion is mainly regulated by the autonomic nervous system(13,14).Saliva volume and biochemical composition differ among individuals; these parameters are influenced by age, sex, and diet.

### Saliva and COVID-19

Saliva can host several viruses including SARS-CoV-2, the transmission chance of viruses through saliva, particularly those causing respiratory infections, is unavoidable in a dental office(15).

The presence of SARS-CoV-2 in saliva may be related to different sources such as i) virus entry to the oral cavity from lower/upper respiratory tract, ii) access to the mouth via oral cavity-specific crevicular fluid or iii) release of viral particles in the oral cavity via salivary ducts from the infected salivary glands (16) Fig.(1).

Based on experience in combating the COVID-19 outbreak, stopping disease transmission by saliva in the dental clinic is vital to the safety of doctors and patients(15 ).The analysis of saliva in COVID-19 cases can help to explain the pathogenesis because epithelial oral cavity cells demonstrated ample expression of the Angiotensin-Converting Enzyme 2 (ACE2) receptor that plays a critical role in allowing SARS-CoV-2 to enter the cells(17).

ACE-2 is a critical COVID-19 receptor. Liu et al. studied SARS-CoV and showed that epithelial cells of salivary gland having elevated ACE-2 expression were infected(18).The ACE-2 expression in minor salivary glands was greater than that in the lungs, indicating that a target for COVID-19 may possibly be salivary glands. Furthermore, before lung lesions emerge, SARS-CoV RNA can be found in the saliva. This could account for asymptomatic infections. For SARS-CoV, the salivary gland is a significant reservoir of the virus in saliva. The positive rate of COVID-19 in the saliva of patients can exceed about 92%, and the live virus can also be cultivated through saliva samples. This proposes that COVID-19 spread through asymptomatic infection may come from the contaminated saliva. Consequently, the source of asymptomatic infection could be salivary glands(19).

SARS-CoV-2 might induce acute sialadenitis and associated symptoms, such as pain, discomfort, inflammation, and secretory dysfunction in salivary glands. SARS-CoV-2 can attach to ACE-2 receptors on the epithelium of salivary glands, fuse with them, replicate, and lyse cells to trigger apparent signs and symptoms, such as discomfort, inflammation, and pain in major salivary glands. After the cytolytic activity of

SARS-CoV-2 lyses the acinar cells, salivary amylase is unleashed into the peripheral blood. It can be inferred that the amylase rises in peripheral blood during the early contamination process. Secreted inflammatory cytokines facilitate the inflammatory reaction that destroys the tissue of the salivary glands as the immunopathological process continues. Granulation and fibrogenesis can restore the inflammatory damage by decreasing immunoreaction. After the severe stage, the function of salivary glands can be anomalous due to contamination with SARSCoV-2, which may induce chronic sialadenitis (20).

### **Hypo salivation**

The salivary gland secretion is dependent on several factors, including temperature, circadian rhythm and intensity and type of chemosensory, masticatory, or tactile stimulation. Hypo salivation, the reduction of un stimulated salivary flow rate, is a common finding in patients mainly reported as a consequence of the use of medication and psychological processes.

Two possible explanations for enhancing the incidence rate of this infection are as follows:

- Lowered saliva secretion can disrupt the oral and airway mucosal surfaces as a physical barrier, thereby enhancing the viral colonization and adhesion.
- This decrease may also hinder the secretion of antimicrobial peptides and proteins(21,22).

Dry mouth was shown to be manifested in a relatively high proportion of COVID-19 patients. Hypo salivation as responsible for exposing patients to a higher risk of getting COVID-19 once the presence of many proteins with antiviral properties in saliva could be reduced. Interestingly, the SARS-CoV-2 infection is more severe in individuals over 50 years of age and with the presence of associated comorbidities such as diabetes, cardiovascular problems and diseases involving the nervous system. It is known that salivary flow reduces with age and is not explained based on

medications used by older adults. Besides, diabetes and medications for systemic disorders have also been associated with hypo salivation. It is known that infectious and inflammatory processes might also lead to hypo salivation. Thus, the possibility of qualitative and quantitative disturbances in saliva secretion by SARS-CoV-2 infection in the salivary gland should not be discarded(23,24).

### **Salivary Liver Function Markers, and COVID-19**

Liver is one of the largest organs in the body. It has many important metabolic functions. Liver tissue has a relatively large amount of enzymes activity and alteration of various enzymes in hepatitis(25).

Liver Function Tests (LFTs) are commonly used in clinical practice to screen for liver disease. The most common LFTs include the serum aminotransferases (ALT, AST), alkaline phosphatase total protein (TP), and albumin (26). Measurement of serum aminotransferases, such as ALT and AST serve as a marker of hepatocytes injury. ALP,GGT act as markers of biliary function and cholestasis whereas TP, albumin reflect liver synthetic function(27).

### **Possible mechanisms for liver damage caused by COVID-19**

1. Angiotensin-converting enzyme 2 is the key receptor for the entry of SARS-CoV-2 into the cells which expressed in both liver cells and bile duct cells and the direct binding of SARS-CoV-2 to ACE2 receptors in cholangiocytes might result in the liver damage due to the damage to bile duct cells(28,29).
2. Systemic inflammatory reaction due to a cytokine storm. The excessive immune response could likely results in injury to the liver resulting in derangement of biochemical markers of liver, individuals suffering from COVID-19 were detected with high levels of TH17, CD8 T cells,

interleukin-2, interleukin-6, interleukin-7, interleukin-10, tumor necrosis factor-alpha (TNF-alpha), granulocyte-colony stimulating factor, interferon-inducible protein 10, high levels of C- reactive protein in samples of their peripheral blood triggering dysfunction of liver enzymes(30,31).

3. Certain medications are hepatotoxic and can cause liver injury during COVID-19 treatment(32).

Lactate dehydrogenase (LD, EC 1.1.1.27: 134 kDa) is a cytoplasmic hydrogen transfer enzyme that involved in anaerobic glycolysis catalyzes the oxidation of L-lactate to pyruvate with the mediation of NAD<sup>+</sup> as a hydrogen acceptor, with the reaction being reversible, it comprises two separately enclosed subunits, resulting in five isozymes. Each isozyme is expressed in a specific organ(33,34). LDH serve as a non-specific indicator of cellular death and associated with worse outcomes in patients with viral infections(35). Suppression of LDH has anti-inflammatory effects due to the down regulation of several inflammatory mediators including cytokines and NO(36).

Albumin is a single chain is a globular protein with a molecular weight of 66 kDa made of 585 amino acids is synthesized by the liver is found in high concentrations in the intestine, muscle, skin, and all body fluids. Albumin interacts with many endogenous and exogenous molecules. which represents more than 50% of the serum proteins and represents an important component of interstitial fluid, whose major function is general binding and transportation of protein, It is the main negative secretory acute phase protein with antioxidant property. This protein is regarded as a serum ultra-filtrate to the oral cavity and it may diffuse into the mucosal secretions and thus found in saliva(37-39).

### **Cytokines**

Cytokines are a family of low-molecular weight, small soluble polypeptides play a critical role in mediating inflammatory

processes released by specific cells that prove to be effective in very low concentrations. Their actions may play out locally or systemically, either on the every cell that secretes the cytokine (autocrine) or on a nearby cell (paracrine). Cytokines are produced through a cascade process in which, one cytokine stimulates its target cells to make additional cytokines. Cytokines are also able to act in a synergistic (two or more cytokines acting together) or antagonistic manner (cytokine causes opposing activities). Multiple cytokines operate as a network in a pleiotropic (that is, the ability of one cytokine to act on different cell types) and redundant (refers to the property of multiple cytokines having the same functional effectors). Cytokines are known to have a short half-life that ranges from a few hours to a few days, with a low concentration of plasma ensuring that it acts for a limited period of time only and thus across short distances(40,41,).

There are two categories of cytokines: one is pro-inflammatory cytokine such as interleukin-1 (IL1), IL6, IL8, and tumor necrosis factor, the other is anti-inflammatory cytokine such as IL2, IL4, IL10, and IL13(42). IL-10 is a pleiotropic cytokine known for its immunosuppressive properties. with a homodimer structure and molecular weight of 37 KDa. Each monomer consists of 160 amino acids with a molecular weight of 18.5 KDa. IL-10. Its gene in humans is located on chromosome 1q31-1q32. Its primary role is to suppress immune activity by blocking pro-inflammatory cytokines synthesis such as TNF- $\alpha$ , TNF- $\gamma$  and IL-1. Various cell populations are able to produce IL-10. In addition to T-cell group (T helper2, T cytotoxic2), monocytes, macrophages and several other cell types such as B cells and keratinocytes may produce IL-10. But the major source of IL-10 production, in studies, is macrophages. IL-10 is effective on different cell populations, especially circulating and tissue resident immune cells as well as keratinocytes(43,44).

The anti-inflammatory IL-10 was as the master regulator of cytokines, found to inhibit the monocyte inflammatory response directly and negatively regulate

the cascade of pro-inflammatory cytokines including TNF- $\alpha$ , IL-6, and IL-8, which induced monocyte hypo-responsiveness in multiple organ dysfunction. It is reported that coronavirus infection, its rapid replication, as well as the delayed type I interferon (IFN-I) signaling activate inflammatory monocyte-macrophage, resulting in an increased cytokine concentration, vascular leakage and pathogenic T cell response(45,46).

Interleukin-6 is a multifunctional inflammatory cytokine, and has been referred to as one of the chief mediators of the acute-phase response, produced by a specific variety of cells, including adipocytes, smooth muscle cells, fibroblasts, lymphocytes and macrophages and endothelial cells in response to LPS and it has both pro inflammatory and anti-inflammatory roles, It is involved in inflammatory, regenerative, metabolic, and neural processes that plays an important role in the response to environmental stress and has been implicated in the pathogenesis of many chronic diseases(47-50).

Interleukin-8 is a pro-inflammatory polypeptide belonging to the member of the C-X-C motif (CXC) subfamily of chemokines superfamily, it is a potent chemo attractant and activator of neutrophils, characterized by the presence of two cysteine residues separated by an intervening amino acid in the first three positions, and is secreted by several cell types, including adipocytes, monocytes / macrophages, T-lymphocytes, endothelial and epidermal cell (51,52).. IL-8 is involved in the initiation and amplification of acute inflammatory reaction; it is secreted by several cell types in response to inflammatory stimuli(53).

Interleukin-8, which can be expressed in vascular endothelial cells, fibroblasts, monocytes and epithelial cells, can mediate a series of cascade reactions stimulating the migration of white blood cells, the formation of neutrophil peroxide, as well as lysosome release, activation and chemotaxis. IL-8 is a potent pro-inflammatory cytokine playing a key role in the recruitment and activation of neutrophils during inflammation(18), and, given the frequent neutrophilia observed

in patients infected with SARS-CoV2, it is possible that IL-8 contributes to COVID-19 pathophysiology.

Healthy tissues have insignificant amounts of IL-8 but its concentration quickly reaches 10-100 times its baseline level in response to pro-inflammatory cytokines such as TNF- $\alpha$  or IL-1 $\beta$ , bacterial or viral products, and cellular stress(54).IL-8, which can be expressed in vascular endothelial cells, fibroblasts, monocytes and epithelial cells, can mediate a series of cascade reactions stimulating the migration of white blood cells, the formation of neutrophil peroxide, as well as lysosome release, activation and chemotaxis.

Tumor necrosis factor  $\alpha$  or TNF- $\alpha$  (which was known formerly as cachexin or cachectin)is an adipokine involved in systemic inflammation and it is a member of a group of cytokines that stimulate the acute phase reaction. It is produced chiefly by activated macrophages, although it can be produced by many other cell types such as CD4+ lymphocytes, natural killer cells, neutrophils, mast cells, eosinophil, and neurons. The synthesis of human TNF $\alpha$  starts with the elaboration of a prohormone, a 26 kDa membrane associated form, which either serves as a precursor for the soluble molecule or binds without processing to the TNF- $\alpha$  receptors through cell-to-cell contacts. This cytokine is synthesized primarily by monocytes/macrophages, although diverse studies have demonstrated that intrinsic renal cells, including glomerular, mesangial, endothelial, and tubular cells, are able to produce inflammatory cytokines that play a role in controlling growth, biosynthetic activities, and functions of cells(55).

### **Salivary biomarkers of oxidative stress**

Reactive oxygen species (ROSs) is a term used to describe small oxygen-containing molecules that are reactive or give rise to reactive species. The name “reactive oxygen species” is preferable to “free radical species”, since not all free radical oxygen species are very reactive, and some non-free radical oxygen species are very reactive, e.g., singlet oxygen(56).

Oxidative stress is caused by the imbalance in the production of ROSs and reactive nitrogen species (RNSs) and the antioxidant molecules in the body, favoring an ROS-rich environment and/or reduced antioxidant reserves(57,58).

Reactive oxygen species are produced during viral infections and significantly affect both the production of oxidizing agents and the synthesis of antioxidant enzymes. Viral infections affect the production of mitochondrial ROS because viruses can induce or inhibit various mitochondrial processes in a highly specific way so that they can replicate and produce progeny(59,60). ROS accumulating at the site of inflammation can enhance pro inflammatory responses by activating the inflammatory signal pathways(61).

Overwhelming production of ROS resulting in oxidative stress is a major cause of local or systemic tissue damage that leads to severe COVID-19. It increases the formation of neutrophil extracellular traps (NETs) and suppresses the adaptive arm of the immune system, i.e. T cells that are necessary to kill virus-infected cells(62).

Antioxidants are capable of reducing oxidative stress by scavenging free radicals(63). In the body anti-oxidative system divides into enzymatic and non-enzymatic categories among which the former includes superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione (GSH), Catalase (CAT), and other variables such as bilirubin, sexual hormones, melatonin, co-enzyme Q, uric acid and nutritional antioxidants (vitamins C, E, beta-carotene and flavonoids ) (64). Infection with COVID-19 leads to excessive activation of monocytes/macrophages which are inflammatory cells responsible for producing the majority of oxidants in the lungs by releasing cytokines and regulating cell adhesion molecules with the development of a cytokine storm(65,66).

### **Methods:**

**Search Strategy** An electronic search was performed on PubMed/Medline,

EMBASE, and Google Scholar for studies comparing biochemical markers in the saliva of patients with COVID-19. The literature search was performed using keywords “COVID-19,” “Liver,” “Aspartate Aminotransferase (AST),” and “Alanine Aminotransferase (ALT), Lactate dehydrogenase, Interleukin-6, Interleukin-10, Tumor necrosis factor-alpha, Malondialdehyde, Superoxide dismutase, Glutathione in various combinations of “AND / OR” from December 1, 2020, till April 15, 2021. Further attempts were made to identify articles by manually searching the references used in these articles.

### Study Designs:

A total of 9 articles were identified by the initial filtering search. We undertook a systematic review and meta analysis investigating abnormal salivary liver tests ,oxidative stress marker, cytokine dysregulation in patients diagnosed with Covid-19.

A full-text assessment of the shortlisted articles for eligibility criteria identified four articles. The following information was extracted: country of study, study setting [saliva collection, study design, sample size, the definition and the prevalence of abnormal liver tests], and relationship with the outcome.

### Results:

Overview of the included studies

A total of 9 articles were included in this review, characteristics of these articles are included in Table (1).

A cross-sectional study by Han, Seong, Kim, et al. (67), Fan Z et al(68)., and Cheng et al(69).looked at the prevalence of liver test abnormalities in COVID-19 positive patients. Abnormal liver function was defined as any parameter (ALT, AST, ALP, GGT, and total bilirubin) more than the upper limit of the normal lab reference value.

Overall, elevations in salivary TNF- $\alpha$ , CRP, IL-6 and,IL-10 levels among patients with Covid-19 were identified in all included studies(). [Yoon *et al*(70)., Chau *et al*(71).and Bosworth *et al*.

Bosworth *et al*(72). Azzi, Carcano, Gianfagna, *et al*(73)and Han, Seong, Heo, *et al*(74)].and Zhang *et al*. (75). reported that the severity of the disease was correlated with increased salivary level of MDA, whereas decrease levels of SOD, catalase, GPX.

### Discussion

#### Salivary Liver Function Markers, and, COVID-19

The main mainly a mitochondrial enzyme is AST. Although its elevated level in the serum is not specific of the hepatic disorder, AST is used mainly to diagnose and to verify persistent cellular injury with other enzymes like ALT(76) .

In this systematic review and meta-analysis, we demonstrate that the salivary levels of ALT and AST increased due to systemic hyper inflammatory state which is the main responsible mechanism caused by drug hepatotoxicity, cytokine storm and/or pneumonia-associated hypoxia, could partly be due to an increased leakage from the damaged cells of soft tissues of peridontium into saliva and a reflection of metabolic changes in the inflamed gingiva(77) .

Both GGT and ALP were considered as “cholangiocyte-related enzymes”. ALP is present in a variety of tissues but it is especially abundant in the liver, bone, and kidney while GGT is mainly distributed in the cell membranes of many tissues including kidneys, bile duct, pancreas, gall- bladder, spleen, heart, brain, and seminal vesicles, it is mediates glutathione uptake and thought to be linked to oxidative stress and chronic inflammation. Hence, for bile duct injury, ALP is more sensitive than GGT(78,79) . Increases in GGT activity can be a response to increase oxidative stress, facilitating increased transport of GSH precursors into cells, GGT is leaked into the serum possibly as a result of normal cell turnover and cellular stresses(80) .

Our study discloses that the salivary LDH levels were significantly higher in COVID-19 patients when compared to the controls. Due to release greater amounts of LDH in the circulation and cytokine-mediated tissue damage(81) . The increase

of LDH reflects multiple organ injury (tissue / cell destruction) and is regarded as a common sign of tissue/cell damage, LDH can convert pyruvate to lactate and might be the key enzyme for pneumococcal pyruvate metabolism and thus pneumococcal survival in blood suggesting viral infection or lung damage, such as the pneumonia induced by SARS-CoV-2 (82,83).

Lactate dehydrogenase is not only a metabolic but also an immune surveillance prognostic biomarker, its elevation is harbinger of negative outcome in immunosuppressive patients(84). LDH increases production of lactate, leads to enhancement of immune-suppressive cells, including macrophages and dendritic cells (DCs), and inhibition of cytolytic cells, such as natural killer (NK) cells and cytotoxic T-lymphocytes (CTLs). LDH is often induced upon T cell activation and proliferation(85). Albumin is one of the major proteins found in the human body that serves as a strong anti-inflammatory agent. It binds with the ROS and RNS produced during inflammation saving cellular injury, when the serum albumin range decreases, cells produce high levels of oxygen radicals with resulting uncontrolled activation of the cells leading them to their death (17).

Albumin bonds to ligands by a reversible process where the equilibrium between bound and unbound depends upon the relative concentrations and depends upon ligand. All ligands capable of binding including proteins fatty acids and indeed the SARS-CoV-2 virus are bound in competition for sites to bind upon the albumin molecule as other viruses (43). Albumin is often used as a marker for the degree of mucositis or inflammation in the oral cavity. Decreased salivary concentrations of albumin may reflect albumin extravasation as a consequence of increased capillary permeability due to due to capillary leakage, malnourishment, inflammation, hepatocellular injury, and renal losses (59,64).

### **Salivary Cytokine Markers, and, COVID-19**

C-reactive protein is produced by the monocytes of the tissue factor classified as

an acute phase reactant, regulated by pro-inflammatory cytokines, like IL-6 and TNF- $\alpha$ , it is an inflammatory marker which is typically not detected in the blood unless some degree of inflammation is present in the body (77). Salivary levels of hs-CRP were measured as a marker for systemic inflammation can contribute to inflammatory responses by activating complement proteins, which can lead to an increase in the production of thrombogenic components, which are bound to the membranes of injured cells(71) were higher in with COVID-19 compared to systemically healthy controls may be due to the reflection of the chronic low grade inflammation(35,44-46). The pathophysiological hallmark of COVID-19 is the severe inflammation and chemokine storm, which explains the elevation of IL-6(70).

Circulating IL-6 concentrations have been closely associated with the clinical severity of COVID-19. salivary IL-6 levels were also found to be significantly elevated in patients with COVID\_19 because the SARSCoV-2 can damage the blood-brain barrier, invade the nervous system through the slow cerebral microcirculation, which the facilitates the interaction between the protein S (spike) and the ACE-2 receptors expressed on the capillary endothelium, and interact with the ACE-2 receptors expressed in target cells(64).

Interlukin-10 is highly abundant, especially during the adaptive immune response. IL10 is likely up regulated to counter overwhelming infection during SARS-CoV-2 infection, but it may be also involved in the infiltration of inflammatory cells and lung fibrosis. However, IL-10 is also known to introduce anergy or non-responsiveness of T-cells in antitumour cell response as well as in viral infection. Blockade of IL-10 using antibody against IL-10 or its receptor or genetic removal of IL-10 resulted in elimination of infection by virus or bacterial pathogen(50). IL-10 blocking, alone or in combination with programmed cell death protein 1 (PD-1), is promising for reinvigorating exhausted T cells and may control COVID-19 pathogenesis. Despite the benefits, there



are still disadvantages, such as the development of chronic inflammatory disorders, thus more experimental studies should be done to clarify whether over activation or ablation of IL-10 could be helpful for severe COVID-19(3-7) .

Interlukin-10 might also amplify the viral sepsis related hyper inflammation observed in some severe/critically ill COVID-19 patients. Because IL-10 directly expands cytotoxic effector CD8+ T cells in human studies, hyper activation of adaptive immunity in COVID-19 patients might contribute to exacerbating disease severity(1,68) .

Tumor necrosis factor level is lower in patient group than the controls, a possible explanation for the decrease in salivary TNF activity may be mediated by increase chemokine and scavenger receptor expression(25-28) .

#### **Salivary biomarkers of oxidative stress, and COVID-19**

Malondialdehyde is a stable produced as a byproduct of polyunsaturated fatty acid peroxidation and arachidonic acid metabolism and is highly reactive. Its levels said to be indicative of the involvement of free oxygen radicals in tissue damage. Free oxygen radicals might cause the lipid peroxidation of biological membranes through a chain reaction. The first step is the initiation reaction, which begins by the removal of hydrogen atom from polyunsaturated fatty acid (PUFA) by free oxygen radicals. The second is the propagation, which culminates in the final step of termination. MDA readily combines with several functional groups on molecules including proteins, lipoproteins, and DNA. The extent of LPO has often been determined by the thiobarbituric acid (TBA) test, which has also been considered for the detection of MDA(50-53) .

The increased leukocyte count is linked to cell activation, as the activated neutrophils are the important sources of oxygen metabolites, which may trigger oxidative modifications in plasma constituents and in cell membranes, which probably accounted for the significant rise in plasma lipid peroxidation along with a significantly reduced antioxidants, clearly

reveals the development of an oxidative stress conditions(72) .

The excess production of oxygen leads to generation of ROS, which may initiate the peroxidation of lipids in cell membranes(50,54) . The reason why MDA level is high is still obscure, but some believe that prostaglandin`s activity is the cause, hypoxia, myocardial ischemia and plate let aggregation leads to rising in the releasing rate of the prostaglandin(40)

Peroxidation of lipids is a chain of reaction which is catalyzed in vivo by heme compounds and lipoxygenases found in platelets, leucocytes, etc., Lipid peroxides formed in this reaction degraded to form a characteristic product such as MDA. Peroxidation provides continuous supply of the radicals which causes damage to tissues in vivo causing atherosclerosis, cancer, inflammatory disease, aging, etc (39). Oxidative storm with all he deleterious effects of reactive oxygen and nitrogen species, notably lipid peroxidation and proteins oxidation of membranes which can contribute to the transformation, hyalinization of pulmonary alveolar membranes with lethal respiratory distress(46) .

Superoxide dismutase is the first detoxification enzyme and most powerful antioxidant that reflects the capacity of the cell to scavenge free radical. enhances the breakdown of superoxide anion into oxygen and hydrogen peroxide. SOD is regarded as the main intracellular antioxidant defense against free radicals (31) . The decreased SOD level may due may be due to compensatory efforts generated by SOD to combat oxidative stress to which patients were chronically exposed. SOD acts as a scavenger for toxic superoxide radical which is implicated in lipid peroxidation. Reduced activity of SOD could be the result of intermolecular and intra molecular cross-linking of proteins and thereby causing conformational changes in SOD, which leads to accumulation of reactive oxygen species such as H<sub>2</sub>O<sub>2</sub>, which induces further lipid peroxidation(32) .

Our study revealed decreased level of GPx and SOD in patients with CONID-19 in comparison with control group. Lowered activity of these enzymes might

be explained by the depletion of antioxidant defense system following the over generation of free radicals. Accumulation of hydrogen peroxide may be one of the explanations for decreased activity of this enzyme (21).

Glutathione is the mother of all antioxidants, the master detoxifier and maestro of the immune system. Glutathione peroxidase is a selenium containing antioxidant, is active fundamentally in the cytoplasm of the cells and only about 10% of its activity takes place in the mitochondria. It catalyzes the decomposition of hydrogen peroxide formed by SOD into oxygen and water, and promotes the activity of GSH, which catalyzes the decomposition and detoxification of lipid peroxides (23-27).

Catalase is the scavenging enzymes that guards the cells from hydrogen peroxide generated within them. It plays a role in tolerance acquisition to oxidative stress in the adaptive response of cells(24) . A decrease in the activity of CAT could be due to increase in the lipid peroxidation product, malondialdehyde which can form cross links, thereby inactivating several membrane bound enzymes (26,30).

## Conclusions

1. In the present meta-analysis we provide evidence that saliva tests are a promising alternative to nasopharyngeal swab tests for COVID-19 diagnosis. Optimized and validated saliva assays offer the possibility of reliable self-collection of samples for COVID-19 testing in the future.
2. Salivary liver biochemical parameters were strongly correlated

with COVID-19 mortality. Measurement of these Salivary liver biochemical parameters might assist clinicians to evaluate the prognosis of COVID-19.

3. We found that elevated salivary levels of oxidative stress and reduction of antioxidant indices can aggravate disease's severity in hospitalized patients with Covid-19.

## Recommendations:

1. Clinical studies with larger patient populations that measure recordings at different stages during the disease are still necessary to confirm the accuracy of COVID-19 diagnosis with saliva. Nevertheless, the utility of saliva as a diagnostic tool opens the possibility of using rapid and less invasive diagnostic strategies by targeting bioanalytes rather than the pathogen.
2. Larger studies are needed to evaluate the relationship between derangement in salivary biochemical and mortality in COVID-19.
3. According to many studies on the role of oxidative stress in the pathophysiology of Covid-19 disease, it seems that further investigations should be conducted to determine the time of onset of antioxidants and their required dose to treat this disease.

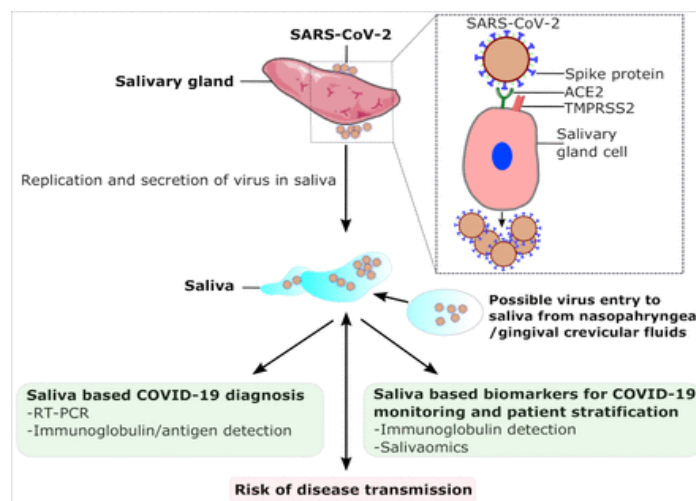


Fig. (1): Schematic illustration demonstrating clinical implications and various sources of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in saliva.

Table (1): Characteristics of the 9 articles.

|   | Reference  | Sample Type            | Article title  |
|---|--|------------------------|--|
| 1 | Han, Seong, Kim, <i>et al.</i> 2020                  | Saliva (not specified) | SARS-CoV-2 was detected in saliva of patients with COVID-19.increased liver disease( AST,ALT)                |
| 2 | Fang <i>et al.</i> 2020 (China)                      | Saliva                 | The positive rate of saliva was 78.1% (25/32).   |
| 3 | Cheng <i>et al.</i> 2020 (Hong Kong, China)          | 2013                   | Viral load of $3.3 \times 10^6$ copies/mL $5.9 \times 10^6$ copies/mL (saliva) of 1 patient                  |
| 4 | Yoon <i>et al.</i> 2020 (Korea)                      | Saliva (not specified) | SARS-CoV-2 was detected up to hospital day 6 (illness day 9 for patient 2) from the saliva of both patients. |
| 5 | Chau <i>et al.</i> 2020 (Vietnam)                    | Saliva                 | SARS-CoV-2 RNA was detected in 20/27 (74%) with available saliva:  |
| 6 | Bosworth <i>et al.</i> 2020 (United Kingdom)         | Saliva (not specified) | 5/15 samples positive in saliva..  |
| 7 | Azzi, Carcano, Gianfagna, <i>et al.</i> 2020 (Italy) | Drooled saliva         | SARS-CoV-2 was detected in the first salivary swab of all 25 patients.                                       |
| 8 | Han, Seong, Heo, <i>et al.</i> 2020 (South Korea)    | Saliva(not specified)  | Decrease in viral load in saliva of a neonate 6, 9, and 12d after symptom onset.                             |
| 9 | Zhang <i>et al.</i> 2020 (China)                     | Saliva (not specified) | Of 15 patients with COVID-19 evaluated, 8 (53.3%) had positive saliva  |

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