Effects of Povidone Iodine and Chlorhexidine on the Severe Acute Respiratory Syndrome Coronavirus-2 Viral load in saliva among patients hospitalized with the Corona Virus-19 disease.

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DECLARATION

I, Parina Bhupendra Patel, of the University of Nairobi, Department of Dental Sciences, declare that this Thesis titled, "Effects of Povidone Iodine and Chlorhexidine on the Severe Acute Respiratory Syndrome Coronavirus-2 Viral load in saliva among patients hospitalized with the Corona Virus disease-19" is my original work.

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APPROVAL

This Thesis report has been submitted with our approval as University of Nairobi supervisors.

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DEDICATION

I dedicate this Thesis to the late Dr. Hudson Alumera and my beloved family, the Patel's, who were a formidable pillar of support, encouragement, and good cheer through this epic journey.

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LIST OF ABBREVIATIONS

ARDS - acute respiratory distress syndrome

CHX - Chlorhexidine

COVID-19 - The Coronavirus Disease 2019

Ct - Cycle threshold

DIC - Disseminated Intravascular coagulopathy

FADI - Fellow of Academy of Dentistry International

FICD - Fellow of International College of Dentists

FPFA - Fellow of the Pierre Fauchard Academy

HVE - High Volume Extractor

HIV - Human Immunodeficiency virus

KEMRI -Kenya medical research institute

MERS - Middle East respiratory syndrome

MSc - Master of Science

MOH – Ministry of Health

PG Dip Dent - Postgraduate Diploma in Dentistry

PGD - Postgraduate Diploma

PI - Povidone-Iodine

PPE - Personal Protective Equipment

rRT-PCR - Real-time Reverse-transcriptase Polymerase Chain Reaction

RT-PCR - Reverse-transcriptase Polymerase Chain Reaction

SARS CoV-2 - severe acute respiratory syndrome coronavirus 2

UON - University of Nairobi

W.H.O- World Health Organization

DEFINITION OF TERMS

Aerosol- An aerosol may be defined as a suspension of particles or droplets in the air and includes airborne dusts, mists, fumes or smoke. Suspended particle sizes may range from a few nanometers to hundreds of micrometers in diameter.

Contagious - An infected person capable of transmitting a disease to another person, usually by direct contact.

Coronavirus - Any group of RNA viruses that affect people and animals and cause a variety of respiratory, gastrointestinal, and neurological symptoms. The name describes crown-like spikes protruding from the virus surface and which resemble the sun's corona.

COVID-19 - The disease the new coronavirus causes is called coronavirus disease 2019, or COVID-19 for short.

Droplets - the term droplet is often taken to refer to droplets >5 μm in diameter that fall rapidly to the ground under gravity, and therefore are transmitted only over a limited distance (e.g. ≤1 m)

Epidemic - The rapid and unexpected spread of a disease within a community or region at a particular time.

Incubation period - The time from a person's first exposure until the onset of symptoms.

Infectious - Describes a disease that can be transmitted through the environment; also describes a person or animal capable of transmitting disease.

Isolation - Separating infected from uninfected people to reduce the spread of infection, such as in hospitals where sick patients remain in designated areas away from others

MERS - Middle East respiratory syndrome is a viral respiratory infection caused by Middle east respiratory syndrome-related coronavirus (MERS-CoV)

Pandemic - The global spread of a new disease afflicting a great many people over a whole country or the world. The World Health Organization has officially designated the current outbreak a pandemic.

PCR test - A diagnostic test for virus particles in blood or other bodily fluids. The letters stand for polymerase chain reaction, a process used to detect the virus's DNA.

Quarantine - Separating or restricting movement of people who appear to be healthy but who may have been exposed to an infectious or contagious disease through contact.

SARS-CoV-2 – severe acute respiratory syndrome coronavirus 2 is a strain of coronavirus that causes COVID-19, the respiratory illness responsible for the ongoing COVID-19 pandemic.

Screening - Testing to detect potential health disorders or diseases in people who do to have any symptoms of diseases.

Virus – an infective agent that typically consist of a nucleic acid molecule in a protein coat, is too small to be seen by light microscopy and can multiply within the living cells of a host

ABSTRACT

Background

Coronavirus infection is a current in progress endemic that presents mainly with respiratory symptoms to severe pneumonia with a worldwide fatality rate recorded at 3.4%. The Coronavirus Disease 2019 (COVID-19) has increased the awareness of transmission risks among health care workers. One of the key approaches in minimizing the threat of transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is to reduce the viral load in the saliva of those infected. Considering that patients with SARS-CoV-2 infection can unknowingly spread the virus in the subclinical period the occupations at the highest risk of contracting COVID-19 include dental hygienists, assistants, and surgeons. Dental care professionals are those with the highest exposure to many aerosols from the oral cavity which can prove to be hazardous to other staff members as well as other patients. Despite the use of personal protective equipment, decreasing the salivary viral titers of COVID-19 could be a key approach in reducing transmission. The use of pre-procedural mouthwashes is, therefore, strongly advocated and this study aimed at investigating the influence of Povidone Iodine (PI) and Chlorhexidine (CHX) mouthwashes on SARS CoV-2 positive patients.

Main Objective

To investigate the effect of Povidone Iodine (PI) and Chlorhexidine (CHX) mouthwashes on SARS COV-2 Viral loads in saliva among patients hospitalized with COVID-19.

Study Design and Study area

This was a randomized double blinded clinical trial that was carried out using a hospital-based population, where both the participants and primary investigator were blinded. The mouthwashes studied included PI, CHX in comparison to a control placebo of distilled water.

The study population included adult patients admitted with COVID-19 in selected hospitals in Nairobi County. These were patients with confirmed diagnosis of COVID-19 disease who were admitted to the isolation wards.

Data collection

All adult patients determined to be positive for SARS-CoV-2 and met the inclusion criteria were recruited to participate in the study. A screening form was used to exclude those who do not fit the inclusion criteria after which 92 participants were selected. Sociodemographic data was collected via a questionnaire for these patients.

Participants were then randomly assigned to three groups, Group A (received a Povidone-Iodine mouthwash) Group B (Chlorhexidine mouthwash) and Group C (Control Group) received distilled water. Baseline saliva sample was collected (T0) after which a mouthwash was administered for 30 seconds, after which a second sample was collected after 30 mins (T1). The saliva samples were then transferred in a cool box to the laboratory where a reverse-transcriptase polymerase chain reaction (RT-PCR) assay was done where their cycle threshold (Ct) levels were determined at both T0 and T1 to monitor the viral loads.

Data Management

Data was collected, coded, entered the computer, then cleaned after which it was analyzed using the Statistical Packages for Social Sciences (SPSS) 20.0 for Windows. Descriptive statistics were computed which included measures of central tendency and dispersion for continuous variables. Effects of the mouthwashes on SARS-CoV-2 viral load levels were determined using Chi square test, t- test and ANOVA tests. The level of significance was set at $p \leq 0.05$. Findings were then presented using text, tables, and figures.

Results

Amongst the 92 participants enrolled, 69 (75%) of the saliva samples found detectable levels of SARS-VoV-2 viral loads in saliva. This study showed that rinsing with PI and CHX resulted in better viral load, with 11% and 7.7 % reduction in salivary viral loads respectively for up to 30mins after rinsing. Whereas the placebo (distilled water) group

maintained a 2.2% reduction in salivary viral load, this change was not found to be clinically significant ($p > 0.05$).

However, a statistically significant overall difference in viral loads was only found between Povidone-Iodine (PI) and distilled water (Placebo) ($F=7.635$, $p=0.001$). This infers that PI had a statistically significant effect on the participants' SARS-CoV-2 viral loads post intervention.

This study demonstrated that Povidone Iodine (PI) was effective in reducing SARS CoV-2 viral load after 30 mins of using the mouthwash ($F=9$, p-value < 0.05) at 11.4%.

Conclusion

PI mouthwash is beneficial in reduction of SARS-CoV-2 salivary viral loads.

Recommendations

PI can be used as a pre-procedural mouthwash as well as an adjunct to PPE to help reduce the salivary load of SARS CoV-2 in healthcare settings where saliva exposure is expected, such as dental practices as well as in situations involving close contact between people in domestic and public spaces.

The results achieved suggest that Povidone Iodine (PI) can be useful in making of Mouthwash policy and protocol for COVID-19 prevention and treatment in health care settings.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

With over 674 million cases worldwide, COVID-19 infection was a public health emergency. Early in 2020 the successful development and testing of the COVID-19 infection vaccines was promising but the pathogens ability to constantly mutate and form new strains has brought about challenges. This current endemic had led to significant mortality and morbidity, and it is imperative that dental professionals consider adjunctive protective measures that increases safety for all.

1.1.1 COVID-19 IN DENTISTRY

Since the beginning of the of the outbreak, the risk of transmitting SARS CoV-2 infection amongst dental professionals has been of growing concern. Most dental professionals are in close contact to the oral cavity in addition to continuously being exposed to salivary aerosols that are being generated from the oral cavity which is significantly dangerous [1]. SARS CoV-2 infection has been shown to remain aerosolized for three hours in an experimental study and was shown to persist on stainless steel and plastic surfaces for more than 72 hours[2]. In dentistry aerosols are recognized as particles smaller than 50 μm that can remain airborne and provide a risk of contamination if it settles on surfaces or enters other respiratory tracts. There is no single method that can eliminate the risk of being infected with SARS CoV-2. A study in 2004 has described 3 layers of defense against aerosols[3]. The first layer involves personal protective equipment (PPE), the second is the use of oral antiseptic mouth rinse and the third layer includes the use of a high-volume extractor (HVE). While the first and third layers are well documented and are being attended to, the second layer is lacking in scientific evidence and attention. The risk of aerosol transmission has thereby advocated the use of an antiseptic mouthwash as a pre-procedural rinse as a measure of infection control by several international and local health

authorities. Studies have shown that SARS CoV-2 have been detected in 91.7 % of COVID-19 infected patient saliva with a mean viral load of 3.3×10^6 copies/ml [4].

1.1.2 CORONVIRUS DISEASE

In 2002 a highly pathogenic virus of zoonotic origin emerged in humans and caused fatalities which was the severe acute respiratory syndrome coronavirus (SARS-CoV) posing a new health threat [5]. This was, however, contained. In December 2019 in Wuhan, China emerged the first reports of a respiratory disease causing pneumonia and its causative agent was found to be the same group of viruses[6]. This novel coronavirus was designated as SARS-Cov-2 and was causing worldwide spread of the coronavirus disease 2019 (COVID-19). This disease is now a global threat to public health as was designated by the WHO [7].

Classical symptoms of COVID-19 infection include fever, cough, fatigue, malaise, headache, loss of smell and taste, shortness of breath, sore throat, nausea and diarrhea [8]. Symptoms vary amongst individuals with some developing none while others experiencing from a range of mild to severe. Of concern is the moderate to severe symptoms such that in the moderate range they experience mild pneumonia while those with the severe form suffer from respiratory failure, shock and multiorgan failure which has a high mortality rate currently [9]. As of the 22nd of February 2023 the coronavirus has infected 674million people with 6.86million deaths.

1.1.3 ORAL ANTISEPTIC

The oral cavity is an essential area of study as positive rates of COVID-19 sensitivity tests in saliva can reach up to 91.7%[4,10]. Therefore, rinsing with an antiseptic mouthwash may be a significant tool in reduction of COVID 19 viral loads and aid in reduction of transmission. Studies have shown that therapeutic mouthwashes have been shown to reduce the quantity of viruses and bacteria in the oral cavity[11,12]. According to the American Dental Association, therapeutic mouthwashes have active ingredients that aid in the reduction of bacteria and viruses in dental aerosols[13].

In the 19th century Povidone Iodine (PI) an antiseptic agent was discovered and found to have one of the broadest spectra of action[14,15]. The metabolites of potency include iodine and hypoiodous acid that delivers free iodine. Therefore, one mechanism of action found is the delivery of free iodine that oxidizes amino acids, nucleic acids and cell membranes; and thereby inactivates the microbes[16]. Another mechanism of action is the oxidation of cell surface receptors hence preventing viral attachment to the cell surface receptors.

Chlorhexidine Di gluconate is an aqueous solution that contains not less than 190 g per litre and not more than 210 g per 30 liters of chlorhexidine gluconate. It is a colorless or pale-yellow liquid which is miscible with water and soluble in alcohol. It is a bisbiguanide antiseptic and disinfectant with both bactericidal or bacteriostatic action against a wide range of Gram-positive and Gram-negative bacteria. Chlorhexidine inhibits some viruses and is active against some fungi and is found to be most active at a neutral or slightly acid pH. Mouthwashes, therefore, have chlorhexidine as an active ingredient to reduce mouth infections and dental plaque accumulation[17].this study sought to find out the virucidal activity of CHX owing to its popular use in dentistry.

1.1.4 SALIVA

Saliva is being progressively more used in the last few years for evaluating human health. It constitutes secretions of the salivary glands, gingival crevicular fluid (GCF), desquamated oral epithelial cells, and specific microorganisms [18]. It also comprises of many proteins such as immunoglobulins (IG), mucins, enzymes, metabolites, hormones, and electrolytes. This composition, therefore, enables the uncovering of pathogens in saliva. It has been shown to identify physiological variations that are comparable or even superior to serum as seen in the detection of acute stress with the aid of alpha-amylase or cortisol [19]. Overall, saliva is a fluid of extensive potential in health assessment due to the clinical evidence that it can present and the non-invasive method of its collection as well as being performed by an individual without any major training requirements and with limited equipment or facilities.

1.2 LITERATURE REVIEW

1.2.1 CORONAVIRUS

ORIGIN

Mysterious cases of pneumonia began to be detected in December 2019 in the Hubei province in Wuhan, China. By January 2020 the causative organism was identified as SARS CoV-2 and the World Health Organization (W.H.O) had named the disease as COVID-19 and announced it as a public health emergency of international concern. By mid-January, Italy had confirmed its first two cases of COVID-19 infection that were brought in by tourists from China. By early March 2020 Europe had become an epicenter of the breakout. Since then, the virus has been found in over 210 countries and territories.

The virus is believed to have originated in the Huanan seafood wholesale market in the Wuhan region. Several claims were made on the natural reservoirs of the virus, some included the possibilities of snakes and pangolins, but research has shown that the virus has originated from bats similar to other such respiratory viruses [20,21].

PATHOGENESIS

The coronaviruses are large spherical single stranded RNA viruses of the Orthocoronavirinae subfamily. Clinical forms of the coronavirus that produce generally mild symptoms include the OC43, HKU1, 229E and the NL63 [22]. These first coronavirus that showed severe symptoms was the severe acute respiratory syndrome coronavirus (SARS-CoV) which was called SARS and was seen in Shendu, China [23]. The second seen in Jeddah was called Middle East respiratory syndrome (MERS) related coronavirus in 2012 which saw 774 deaths [24]. And the third was the SARS-CoV-2 that is understood to have originated from bats and transmitted to humans via pangolins who were the intermediate hosts.

The SARS-CoV-2 infection is shown to target nasal epithelial cells, bronchial epithelial cell and pneumocytes. This is achieved by the viral spike protein (S) that binds to the angiotensin-converting enzyme 2 (ACE2) receptor. Hence, this acts as a cellular doorway for the virus. Following attachment, a protease found in the host cell called the type 2 transmembrane serine protease (TMPRSS2) cleaves the ACE2 and thereby promoting viral uptake of the S protein [25]. The S protein is usually the target by the neutralizing antibodies and, therefore, has been an area of ongoing research [25]. On entry the virion uncoats and its genome enters the cell cytoplasm.

TRANSMISSION AND INFECTION OF COVID-19

Transmission of the virus is through direct contact. It can also spread when an infected individual coughs or sneezes and causes aerosol production of particulates containing the virus. If another person breathes or swallows them this can effectively transmit the virus to them. Virus transmission can also occur from touching a surface that contains the virus and then contact transmission with oral, nasal and eye mucous membranes [26]. Therefore, prevention of cross-contamination as well as infection preventive practices such as regular handwashing, wearing of masks and social distancing is crucial in managing this outbreak.

CLINICAL PRESENTATION OF COVID-19

A series of studies have shown that the average incubation period of COVID-19 infection is about 5 days where 97% of those develop symptoms within 11 days of exposure with the virus. Therefore, the quarantine period of 14 days for potentially exposed individuals is advocated [27]. Fever is one of the most common clinical symptoms that has been reported with about 99% of people experiencing it at a point in time of their illness[9]. Other symptoms commonly reported include cough, dyspnea, anorexia, fatigue, myalgia and anosmia[28,29]. Some patients have also been shown to present with gastrointestinal symptoms such as abdominal pain, vomiting and loose stools[30]. Complications seen in patients with COVID-19 infection are due to the ‘cytokine storm’ and can include acute respiratory distress syndrome (ARDS), Acute respiratory failure, Sepsis, Disseminated

Intravascular coagulopathy (DIC), Acute liver and Kidney injury and Pulmonary embolism[31].

COVID-19 IN KENYA

Africa reported its first case of SARS CoV-2 infection in Egypt on the 14th of February 2020. The World Health Organization (W.H.O) declared it a global pandemic on the 11th of March 2020. The initial case of COVID-19 infection was reported in Kenya on the 13th of March afflicting a 27-year-old woman who had travelled from the United States. On the 17th of March the next two cases were reported. By the 4th of April 2020 the Government of Kenya- Ministry of Health services reported 110 positive cases. As of 22nd February 2023 the cumulative number of cases reported were over 343,000 with over 5,000 deaths [32]. Data has shown that the country had seven waves of the pandemic, with the first in July/August 2002, the next peaking in October/November 2020, the third in March/April 2021 and the most recent one in November 2022.

1.2.2 COVID -19 IN SALIVA

To et al in 2020 published two reports using saliva that was collected by asking the patient to cough out saliva from their throat into a sterile container and adding a viral transport medium to the sample. The detection of the virus was by a real-time reverse transcription-quantitative polymerase chain reaction (rRT-PCR) and the range of values detected was from 9.9 × 102 copies/mL to 1.2 × 108 copies/mL[4]. Azzi et al in 2020 also collected saliva through passive drool in patients who were undergoing endotracheal intubation and mechanical ventilation. The collection was performed intraorally by a physician with the use of a pipette[33].

In these conditions, SARS-CoV-2 infection was detected in all saliva samples collected from a group of 25 patients with severe to very severe disease who were already diagnosed by detection of the virus in pharyngeal or bronchoalveolar swabs[33]. Another study collected saliva from a 27-day-old neonate diagnosed with COVID-19 infection reported values in the range of 105 copies/mL that were similar to the values obtained with pharyngeal swabs but lower than those from bronchoalveolar swabs. These studies indicate

that saliva is a good indicator for detecting the viral loads in SARS-CoV-2 positive patients[34].

Using nasopharyngeal and oropharyngeal swabs has limitations such as the discomfort for the patient and the need for the intervention of a healthcare worker in a disease with a high risk of nosocomial transmission [35]. These collection systems can also induce coughing and sneezing, generating aerosol, which can transmit the virus. In addition, in cases of thrombocytopenia or any other coagulation disorder, this procedure can cause bleeding. These drawbacks can limit the use of swabs, especially in serial monitoring or mass test programs. Sputum has been also proposed as a non-invasive lower respiratory tract specimen but 72% of COVID-19 infected patients were not able to produce it for collection [36]. The difficulty of obtaining sputum also has been described in SARS CoV-2 infection, a virus with many similarities with the Covid-2019, especially at early stages of infection when no cough or only a dry cough is present. The current standard in diagnostic testing is a nasopharyngeal or oropharyngeal swab. This technique of specimen collection is quite uncomfortable to the patient and has shown discomfort and bleeding in patients with thrombocytopenia [36,37]

The use of saliva could improve these drawbacks as it has the following advantages: Rapid detection is essential in controlling the spread of SARS CoV-2 infection amongst communities worldwide. If several specimens are needed to monitor viral loads this specimen collection method is not ideal hence saliva comes in as a non-invasive collection method for diagnostic evaluation. Saliva specimens are easy to collect. The patient is asked to spit into a sterile collection container using the passive drool technique. It is a painless non-invasive procedure that is well tolerated by patients and testing can, therefore, reduce the risk of exposing health care workers.

Viral loads in saliva may be detected using cycle threshold (Ct) values. This is the number of cycles required for the fluorescent signal to exceed that specific threshold value. The lower the Ct value, the more RNA is present in the original sample, indicating a higher viral load. The amount of viral RNA present in positive samples is inversely proportional to the corresponding Ct value, meaning that the greater amount of viral RNA, the lower the

Ct value obtained. For SARS-CoV-2, a Ct value of less than 40 is considered a positive result.

The Ct value will help measure viral load progression. For this study the aim was not to eliminate the virus but to reduce the transmission, by reducing the salivary viral load long enough for the dental caregiver to perform their procedures which are averagely an hour long, thereby reducing the risk to the dental professional.

1.2.3 ROLE OF MOUTHWASHES

Certain mouthwashes have ingredients that target lipid membranes of viruses; therefore these antiviral ingredients could play a role in reducing SARS CoV-2 viral loads and reduce transmission of the virus. Thereby in this context PI and CHX emerged as mouthwash agents with potential. They are well reported mouthwashes that have an excellent safety record and no adverse reactions[38].

Although there is still no clinical evidence that the use of mouthwashes could prevent SARS-CoV-2 transmission, the Center for Disease Control and Prevention (CDC) have recommended the use of preprocedural mouthwashes before oral procedures[39].

POVIDONE-IODINE (PI)

Several antiviral in vitro studies have shown PI activity against viruses such as adenovirus, rhinovirus, coxsackievirus and herpes virus[40]. Another in vitro study has shown that PI solutions of as low as 0.23% concentration effectively inactivates SARS CoV and Middle East respiratory syndrome (MERS) with a contact time as low as 15 seconds, and with SARS CoV-2 being of the same family similar results are expected[41,42]. An in-vitro study was conducted and concluded inactivation of SARS CoV-2 following usage of PI with a contact time of 15 seconds, this study however was conducted in a controlled environment and did not mimic any of the conditions which are found in the oral cavity[43]. One of the first in vivo tests by Lamas et al in July 2020 was a case study showing PI was more virucidal amongst patients with higher viral loads of SARS-Cov2. The dose administered was 15ml of 1% for 1 min, a significant fall in viral load was seen that remained for 3 hours[44]. Another recent randomized controlled trial in 16 COVID-19 positive patients

showed that PI reduced viral loads, however, those studied had varied results for CHX. The small sample size may have resulted in the varied results[45].

Considering the above mentioned studies gargling with PI may be an effective method in reducing transmission of SARS CoV-2 infection. The benefit of gargling with PI is advocated in the Japanese clinical respiratory guidelines[46].

CHLORHEXIDINE(CHX)

CHX is considered a gold standard mouth rinse for chemical control of supragingival biofilm. Chlorhexidine gluconate has been shown to be effective against many respiratory viruses such as the Herpes and Human Immunodeficiency virus (HIV). An in vitro study by Jain et al 2021 showed CHX has viricidal efficacy against SARS-CoV-2[47]. However, a smaller clinical trial in Singapore involving 6 patients did not detect any reduction in SARS-CoV-2 viral loads following the use of chlorhexidine mouthwash [48]. While another small study showed 2 cases of salivary viral load reduction after gargling with 15ml of 0.12% CHX. This varying data and small sample sizes call for further investigation of CHX against COVID-19 infection [49].

CHAPTER 2

STATEMENT OF RESEARCH PROBLEM AND JUSTIFICATION

2.1 PROBLEM STATEMENT

It has been established that the oral cavity is a major site of SARS CoV-2 replication and shedding leading to high transmission rates via the oral cavity when an individual coughs and/or sneezes. COVID-19 infection was a worldwide pandemic and a current endemic and has caused over 5.5 million deaths since it began worldwide. Dental practices pose a potential risk of COVID-19 cross-infection amongst patients and health professionals; and uncontrolled spread will lead to considerable morbidity and mortality. With COVID-19 infection being an untreatable disease entity interventional modality to reduce cross-infection can help prevent further progression and spread of the virus. Therefore, assessment of the viral load orally is important to advocate possible agents that reduce viral loads and thus reduce the risk of transmission significantly.

Since SARS CoV-2 enters the human body via the oral cavity and nasopharyngeal passages therefore oral mouthwashes could help fight the virus to a certain extent and this research aims at understanding the influence of mouth rinses on SARS CoV-2 viral loads in the hope that this will result in a reduction in oral viral loads and therefore curb spread of the COVID-19 disease.

2.2 JUSTIFICATION

If mouth rinses are successful in reducing the viral load of SARS-CoV-2 expelled by the carrier this could decrease cross-contamination related to aerosol-generating dental procedures and this can be advantageous to dental professionals carrying out procedures as well as patients. With the continuous emergence of various strains and the mutations of the virus there is a decrease in the vaccine efficacy. Therefore, the beneficial impact of antiseptic mouthwashes could become more relevant due to the evolution of this pandemic,

which suggests that despite the hygienic measures and social distancing, SARS CoV-2 may not be eradicated till 2024[50]. The use of saliva to detect COVID-19 causative agent SARS CoV-2 is more easily accepted by the patients since it is non-painful and non-stressful. Therefore, it can be used in large scale epidemiological studies, and certain populations, such as children. It is also easy, fast, and cost effective to collect, allowing widespread testing.

Several ongoing clinical trials aim at evaluating the effect of the use of pre-procedural antiseptic mouth rinses on SARS-CoV-2 viral load in saliva and other fluids have been found. However, these studies have a very small sample size and therefore the reliability and validity of these studies is questioned. PI and CHX mouthwashes have been studied continuously, and it has been reported that the oral administration of CHX and PI can effectively reduce the number of oral viruses. They are often recommended in clinical practice because it is well documented and accepted and available worldwide. 0.1 % of PI and 0.12% of CHX were used for this study as this are universally accepted concentrations that have been extensively studied.

With several varying results this study aimed to evaluate the efficacy of pre-procedural mouthwash on dental aerosol viral loads. No dental procedure was intended for our study. This study aimed at collecting quantitative data on viral load reduction following the use of 1 mouthwash.

2.3 OBJECTIVES

2.3.1 MAIN OBJECTIVE

To investigate the effect of Povidone Iodine (PI) and Chlorhexidine (CHX) mouthwashes on SARS COV-2 Viral loads in saliva among patients hospitalized with COVID-19.

2.3.2 SPECIFIC OBJECTIVES

1. To determine base line viral loads of SARS Cov-2 in saliva among hospitalized COVID-19 patients before intervention (T0)

2. To determine viral loads of SARS Cov-2 in saliva among these hospitalized COVID-19 patients after the use of PI and CHX mouthwashes and the placebo (T1).

3. To compare the viral loads of SARS Cov-2 in saliva among PI and CHX mouthwash users with that in the control group(placebo).

2.4 HYPOTHESIS

2.4.1 NULL HYPOTHESIS

There is no difference in SARS CoV-2 viral loads change after usage of PI and CHX ($\mu1=\mu2=\mu3$).

2.4.2 ALTERNATE HYPOTHESIS

There is significant difference in SARS CoV-2 viral loads changes after usage of PI and CHX ($\mu1\neq \mu2\neq \mu3$).

2.5 STUDY VARIABLES

Table 1 Study Variables

VARIABLES	**MEASUREMENT**
Sociodemographic variables	
Age	Number of years
Gender	Male or female

Residence	Where the participants currently reside (Nairobi or outside Nairobi County)
Education	Level of education attained: Primary, Secondary, Tertiary
Independent variables	
Vaccination Status	Vaccinated (Type, Number of doses received) Not vaccinated
Presenting Symptoms at time of vaccination	Fever, cough, headache, anosmia, ageusia, nasal congestion, sore throat, muscle pain, diarrhea, difficulty breathing
COVID-19 status (current)	Positive or Negative
Type of mouthwash used	Povidone- Iodine (PI) Chlorhexidine (CHX) Placebo (distilled water)
Dependent (Outcome) variables	
SARS CoV-2 Viral load	Ct Value (cycle threshold)

CHAPTER 3

METHODOLOGY

3.1 STUDY AREA

The area of study was Hospitals within Nairobi County. The hospitals included were Kenyatta National Hospital, Mbagathi County Hospital, Mama Lucy Kibaki Hospital, Coptic Hospital. These hospitals were selected as they had COVID-19 isolation wards present in their facilities.

The laboratory testing was carried out at the Kenya Medical Research Institute within the Centre for Virus Research (CVR) which is located off Raila Odinga Way, Nairobi, Kenya.

3.2 STUDY POPULATION

The study population included SARS CoV-2 positive patients who were 18 years and above and who consented to the study and admitted in the Isolation Wards at Hospitals within Nairobi.

3.3 STUDY DESIGN

This was a randomized double blind clinical trial with two experimental groups and one control group. It was double blinded as the patients and the examiner were both blinded.

3.4 SAMPLE SIZE DETERMINATION

The sample size for each group was determined using a two-tailed t-test as described by Stephen BH et al [51].

The following equation was thus used to determine minimum sample for each group;

$$n = [(1/q1 + 1/q2)\, S^2\, (Z\alpha + Z\beta)^2] \div E^2$$

Where;

α = 0.05 (Margin of error)

β = 0.2 (Probability of failing to reject the null hypothesis under the alternative hypothesis. Type II error rate.)

q1 =0.5 (Proportion of subjects that are in Group 1 (exposed))

q2 = 0.5 (Proportion of subjects that are in Group 2 (unexposed); 1-q1)

E = 0.5 (Effect size (If μ1 = mean in Group 1 and μ0 = mean in Group 2, then E = μ1 - μ0.))

S = 0.5 (Standard deviation of the outcome in the population)

Zα = 1.9600 when α=0.05 (The standard normal deviate for α)

Zβ = 0.8416 when β =0.2 (The standard normal deviate for β)

(E/S) = 1.000 (Standardized Effect Size)

Therefore;

$$n = [(1/0.5 + 1/0.5)\ 0.5^2\ (1.9600 + 0.8416)^2] \div 1^2$$

Hence, the minimum sample for each sample = 17

However, in this study 30 participants were recruited per group.

3.5 SAMPLING METHOD

All adult patient's laboratory determined to be positive for SARS-CoV-2 and admitted in the isolation wards of the referenced hospitals were recruited into the study consecutively until the required sample size was attained.

3.6 INCLUSION AND EXCLUSION CRITERIA

3.6.1 INCLUSION CRITERIA

- Individuals who were above 18 years of age and gave voluntary consent to participate in the study were selected.
- Individuals who were admitted and were positive for SARS-CoV-2 using the RT- PCR test with mild or moderate clinical conditions with no need for intensive care were included.

3.6.2 EXCLUSION CRITERIA

The following patients were excluded from the study population;

- Individuals who were unable to gargle or spit
- Patients receiving anti-viral medications
- Individuals allergic to any of the active ingredients and had a history of allergies to PI or CHX
- Individuals who had thyroid diseases or current radioactive iodine treatment
- Individuals who were in a coma and on a ventilator.
- Female patients who were pregnant or lactating.

3.7 RANDOMIZATION TECHNIQUE AND BLINDING

The participants who meet the inclusion criteria were selected after which they were randomly divided into three groups through a computer-generated random table.

The three groups included;

Groups A- rinsed and gargled with Povidone-Iodine (1%, 15ml)

Group B- rinsed and gargled with Chlorhexidine (0.2%, 15ml)

Group C -Control Group rinsed and gargled with distilled water

Blinding was ensured by an independent person (an assistant) who was responsible for dispensing uniform amounts of the mouthwash into the bottles with code 1,2 or 3 for either PI, CHX and distilled water. The Research assistant maintained a register of randomization

determining which patient got which mouthwash. Neither the subjects nor the primary investigator in the research group were aware of which mouthwash gargle had been administered. The register was monitored and controlled by the assistant.

3.8 PREPERATION AND STORAGE OF MOUTHWASHES

The mouthwashes were prepared by a pharmaceutical company, Laboratory and Allied Limited which is located on Mombasa Road. All mouthwashes were prepared by putting them in amber glass bottles that were identical in appearance, thereby masking any changes in color and consistency. All the oral rinses were flavored with peppermint and a sweetener to mask the taste, including the distilled water. They were then stored in a cool dry place under 30˚C in radiopaque amber glass bottles that were UV protective.

The mouthwashes were then dispensed and stored by the Research assistant at the University of Nairobi- School of Dental sciences premises at the Department of Periodontology.

Figure 1: Photo of prepared mouthwash sample.

3.9 PRELIMINARY PHASE

A preliminary visit was made to the KEMRI laboratory to work out feasibility, logistics and procedures concerning the real-time PCR analysis. A pilot was carried out at the hospital, where the biodata, clinical examination forms and sample collection protocols were carried out using five test subjects. The laboratory tests were then carried out at KEMRI.

3.10 DATA COLLECTION

3.10.1 SOCIODEMOGRAPHIC DATA COLLECTION

Data concerning socio-demographic variables was collected from participants using interviewer administered and serialized questionnaires (Appendix 6) by the principal investigator. The participants age, gender at time of admission was retrieved from the patients' medical records and recorded in the questionnaire form. The form also included the place of residence, level of education and vaccination status. This study was conducted from 2022-2023.

A screening form (Appendix 5) was used to exclude and include participants.

3.10.2 DATA COLLECTION TIMELINE

i. Eligible patients were assigned to one of the three groups, A, B, or C
ii. Baseline (T0) passive saliva samples were collected
iii. Participants then rinsed and gargled with 15ml of the mouthwash for 30 seconds.
iv. The participants were then instructed not to eat or rinse for the next 30 mins. This was easy to control as the patients were in the isolation wards and had fixed mealtimes, so samples were collected between mealtimes.
v. After 30 minutes a second saliva sample was collected (T1)

3.10.3 SALIVA SAMPLE COLLECTION

Prior to saliva sample collection, the participants were asked to be seated comfortably on their beds for a few minutes. They were then asked to slightly lean forward and not to swallow or speak. After about 2 minutes, the saliva that pooled in the anterior floor of the mouth was collected by passively drooling into 50mL pre weighed, airtight, serialized, centrifuge compatible polystyrene tubes. A minimum of 3mL whole saliva sample collection was done passively. This was the baseline saliva sample (T0). Absorbent paper towels were provided for any untoward spillages. Saliva collection was carried out between 8:00 a.m. and 11:00 a.m. this is recommended as posterior oropharyngeal saliva collected in the early morning could increase diagnostic sensitivity. After a night of sleep lying supine, the posterior oropharynx will contain secretions dripping down from the nasopharynx as well as secretions from the lower airways moved up by ciliary motion.
The participants were asked to rinse and gargle with 15ml of the respective mouthwash for up to 30 seconds. T1 was then collected after 30 mins following the same protocol as the baseline saliva sample (T0).

To be able to enroll participants and administer the materials and collect saliva samples from multiple study sites, the principal investigator allocated a day per site enabling them to move around efficiently as per the table below.

Table 2. Study Sites

DAY	**Hospital**
Mondays	Kenyatta National Hospital
Tuesdays	Mbagathi County Hospital
Wednesdays	Mama Lucy Kibaki Hospital

Thursdays	Coptic Hospital

3.10.4 BIOSAFETY AND CONTROL OF CROSS INFECTION

Precautions were taken to protect the participants, the investigators, and others in the research environment from the risk of cross infection.

The investigators wore personal protective equipment (PPE) which KN95 surgical masks, surgical cap, eye wear, face shields, boots, overalls and clean disposable examination gloves, cups and paper towels for sample collection of every participant. The principal investigator thoroughly washed their hands before donning the PPE.

Each study participant was covered with a disposable bib, and the amber glass bottles were adequately disposed of as per medical waste protocol. Pre-packed sterile centrifugation tubes were used to avoid spillage of the saliva and packed into a clean cool box for transportation to the laboratory.

Any inadvertent spillage on surfaces was cleaned and disinfected using hypochlorite swabs. Saliva handling was done under supervision of a laboratory technologist in compliance with biosafety protocols[52]. Waste disposal was done according to hospital guidelines and any used instruments were taken to the central sterilization unit for cleaning.

3.10.5 SAMPLE STORAGE AND TRANSPORTATION

Samples were stored in tubes that were then placed in a sealable polythene bag, covered with ice cubes and gel ice packs inside a cooler box. This was then transported in the shortest time possible (maximum 2 hours) to the laboratory for processing.

3.10.6 LABORATORY PROCEDURE

All the laboratory procedures were done as prescribed in the Abbott Real time manual with respect to biological principles, sample preparations, reagent preparation and reaction plate assembling, amplification, detection, reagents handling and quality control procedures. Additionally controls for negative and positive results were considered ([53]).

3.11 DATA RELIABILITY AND VALIDITY

i. Several measures were put in place to ensure that assessment tools produced stable, consistent, and reliable results. A pilot phase was carried out to ascertain the validity and reliability of questionnaires, examination forms and instruments. Saliva collection protocol was also assessed in the pilot phase

ii. All the equipment and machines used in the study were calibrated on the15/05/22 and passed quality assurance and quality control checks by the laboratory technician at the KEMRI laboratories

iii. Dummy samples were used for a test run before the actual assay to confirm that the analytical procedures employed were suitable for their intended use.

iv. Samples were assayed against standard reagents and in duplicates for reliability and trueness.

v. Repeat tests were carried out at given intervals, every 15^{th} sample to assess reproducibility and validity.

vi. All the standards and reagents were sourced from the same supplier for precision and reproducibility

vii. For inter- examiner reliability, the principal investigator was calibrated by the supervisors who are periodontologists. Inter and intra-examiner reliability was calculated using the Cohen's kappa score, whereby a score above 80% was acceptable.

Minimization of bias and errors;

i. Calibration of the principal investigator was carried out by the principal supervisor, Dr Wetende A., prior to data collection regarding participant examination and saliva sample collection.

ii. A single laboratory technician carried out all the laboratory tests to minimize any error

iii. Calibration of the Abbott m2000sp was done prior to testing of any samples by the laboratory technician

iv. Having one principal investigator

v. One week prior to data collection training was carried out after which a pilot test run was done to streamline data collection, sample collection and sample storage.

vi. The transport and handling of the sample was done in consultation with the same laboratory technician.

3.12 DATA ANALYSIS AND PRESENTATION

Data was entered then cleaned and analyzed. The analysis was done using Statistical Packages for Social Sciences (SPSS) 20.0 for Windows Microsoft- Excel. Descriptive statistics were computed including measures of central tendency and dispersion. Comparison of the effect of Povidone iodine and chlorhexidine was determined using Chi square test, t- test and ANOVA tests. Findings have been presented using text, graphs and tables.

3.13 ETHICAL CONSIDERATION

i. Ethical approval to conduct the study was sought and obtained from the Kenyatta National Hospital and University of Nairobi Research Ethics and standards committee. (Appendix 1)

ii. Approval was sought from the various Hospital for conduction of this study within their premises.

iii. The purpose of the study was explained to the participants in a language they best understood, any queries were answered appropriately, and written and informed voluntary consent was obtained from every participant before commencement of the study as shown in Appendix 3 and 4.

iv. All individuals who meet the inclusion criteria had an equal chance of being included in the study.

v. There was no risk to the participants during clinical examination and sample collection as these were carried using the World Health Organization standard hospital treatment protocols.

vi. All information gathered was protected by a coded numbering system, which was stored in a password protected computer that could only be accessed by the researcher and was treated with the utmost confidentiality.

vii. There were no anticipated financial benefits either to the investigator or the participants in this study.

viii. There was also a provision for participants to withdraw from the study at any point of the study without any dire prejudice.

3.14 STUDY LIMITATIONS

The limitations for this study included:

i. This study was carried out on hospital-based SARS-CoV-2 positive patients, some exhibiting severe symptoms, so participation was not always guaranteed.

ii. Inaccurate data provided by the participants regarding the exclusion criteria could not be ascertained.

3.15 BENEFITS OF THE STUDY

Currently, there is no information on SARS-CoV-2 viral loads in saliva among Kenyan adult populations and Africans at large. The findings of this study may be used to develop chair side pre-procedural mouthwash protocols. This study will add to the body of knowledge in this field regarding reduction in contamination and cross infection of the disease as well as providing information on Saliva and its reliability as a media for SARS CoV-2 testing in a Kenyan population.

The submission of this study is also in partial fulfillment of the requirements for the award of a Master of Dental Surgery in Periodontology Degree for the Principal Investigator.

CHAPTER 4

RESULTS

4.1 SOCIODEMOGRAPHIC CHARACTERISTICS

A total of Ninety Two participants were recruited into the study.

4.1.1 AGE

The age of the participants ranged between 18 – 72 years with a mean age of $\bar{X}$ = 34.14 (SD = 12.8) years (Table 3). The mean age for males were $\bar{X}$ = 36.2 (SD = 15.1) and the mean age for females $\bar{X}$= 32.1 (SD = 9.8).

Table 3: Distribution of Participants by Age (n = 92).

Number of Participants	Minimum Age (Years)	Maximum Age (Years)	Mean Age (Years)	Standard Deviation
92	18	72	34.14	12.8

4.1.2 GENDER

Of the 92 participants, (45, 48.8%) were males while (47, 51.1%) were females (Table 4).

Table 4: Distribution of Participants by Gender (n = 92).

	Male	Female	Total
	n (%)	n (%)	n (%)
Frequency	45 (49)	47 (51)	92 (100)

4.1.3 EDUCATION LEVEL

33% of the participants had achieved primary level education, 37% secondary education while only 22% had completed tertiary education (Table 5).

Table 5: Distribution of Participants by Level of Education

Education Levels	n	%
Primary	33	35.9
Secondary	37	40.2
Tertiary	22	23.9
Total	92	100

4.2 COVID 19 VACCINATION STATUS

Amongst the participants' vaccination status, (39, 42.4%) had received at least one dose while (53, 57.6%) had not been vaccinated against SARS-CoV-2 (Table 6). The most common vaccine administered was AstraZeneca (18, 46.2%), followed by Moderna (12, 30.8%) and Pfizer (7, 17.9%) with Johnson and Johnson (2, 5.1%) being the least administered vaccine. Out of the 53 vaccinated participants, majority (26, 66.7%) got 1 dose of vaccination while (13, 33.3%) got 2 doses of vaccination. None of the vaccinated participants had gotten booster vaccinations.

Table 6: Distribution of Participants by Vaccination status

Vaccination status

Characteristics		Total	
		n	%
Vaccinated	Yes	39	42.4
	No	53	57.6
Vaccine type	AstraZeneca	18	46.2
	Moderna	12	30.8
	Pfizer & JnJ	9	23.1
Doses	1	26	66.7
	2	13	33.3
	Booster	0	0

The differences in distributions of vaccination characteristics by age groups were not statistically significant (x^2= 1.392, p> 0.05). This infers that there were no statistically significant associations between the various vaccination characteristics and age groups (Table 7).

The difference in distribution of vaccination status by gender was statistically significant (x^2= 4.319, p< 0.05). This infers that there was a statistically significant association between the participants' vaccination status and gender (Table 7).

The differences in distributions of vaccination characteristics by education levels were non-statistically significant (x^2= 0.333, p> 0.05). This infers that there were no statistically significant associations between the various vaccination characteristics and participants' education levels (Table 7).

Table 7: Distribution of Participants by Socio-demographic characteristics and Vaccination status (n = 92).

		Vaccinated			
Variable		**Yes**	**No**		
		n (%)	**n (%)**	**χ2**	**P**
Age (years)	< 30	19(20.7)	25(27.2)	χ2 = 1.392	.499
	31-45	11(12.0)	20(21.7)		
	>45	9(9.8)	8(8.7)		
Total		**39(42.4)**	**53(57.6)**		
Gender	Male	24(26.1)	21(22.8)	χ2 = 4.319*	.038
	Female	15(16.3)	32(34.8)		
Total		**39(42.4)**	**53(57.6)**		
Education levels	Primary	13(14.1)	20(21.7)	χ2 = 0.333	.847
	Secondary	17(18.5)	20(21.7)		
	Tertiary	9(9.8)	13(14.1)		
Total		**39(42.4)**	**53(57.6)**		

Chi square test was used.

The distribution of vaccine type and doses by education levels can be further seen in tables in the Appendix 7.

4.3 MOUTHWASH GROUPS

Among the 92 participants, 31 (33.7%) participants were assigned PI (1%), 31 (33.7%) assigned CHX (0.2%) and 30, (32.6%) participants were assigned distilled water (placebo) (Table 8)

Table 8: Distribution of participants by type of mouthwash (n = 92).

	Mouthwash Agent	PI (1%)	CHX (0.2%)	Placebo	Total
	n	31	31	30	**92**
Total	%	33.7	33.7	32.6	**100**

4.3.1 DISTRIBUTION OF PARTICIPANTS BY AGE AND TYPE OF MOUTHWASH ADMINISTERED

The differences in distributions of mouthwash agents by age groups were non-statistically significant ($\chi 2$=2.858, p>0.05). This infers that there was no statistically significant association between the mouthwash agents and participants' age (Table 9).

Table 9: Distribution of participants by type of mouthwash and age groups (n = 92).

	Age group (years)					
	18-30	31-45	>45	Total		
Mouthwash agent	n (%)	n (%)	n (%)	n (%)	$\chi 2$	p
PI (1%)	14(15.2)	9(9.8)	8(8.7)	31(33.7)	2.858	.582
CHX (0.2%)	15(16.3)	10(10.9)	6(6.5)	31(33.7)		
Distilled Water	15(16.3)	12(13.0)	3(3.3)	30(32.6)		
Total	44(47.8)	31(33.7)	17(18.5)	92(100)		

Fishers exact test of association was applied.

4.3.2 DISTRIBUTION OF PARTICIPANTS BY GENDER AND TYPE OF MOUTHWASH ADMINISTERED

The difference in distribution of participants by Gender and type of Mouthwash administered was not statistically significant (x^2= 0.154, p> 0.05). This infers that there was no statistically significant association between the mouth rinse agents and participants' gender (Table 10).

Table 10: Distribution of participants by type of mouthwash and gender (n = 92).

	Gender				
	Male	Female	Total		
Mouth rinse agent	n (%)	n (%)	n (%)	$\chi 2$	p
PI (1%)	16(17.4)	15(16.3)	31(33.7)	0.154	.926
CHX (0.2%)	15(16.3)	16(17.4)	31(33.7)		
Distilled Water	14(15.2)	16(17.4)	30(32.6)		
Total	45(48.9)	47(51.1)	92(100)		

Chi-square test of independence was applied.

4.4 VIRAL LOAD LEVELS AT BASELINE

The Ct (cycle threshold) value is the number of polymerase chain reaction cycles at which the fluorescence signal of a particular sample crosses the defined threshold. Ct value has an inverse relationship with the amount of virus present in a particular sample. A higher Ct value indicates a lower viral load in the sample and vice versa. Doubling of viral load results in a single point difference in Ct value. For SARS-CoV-2, a Ct value of less than 40 is considered a positive result. The Ct value will help measure viral load progression.

This study found that the SARS-CoV-2 viral levels were detected in 75%(n=69) of the saliva samples. Amongst the 92 enrolled and randomized patients, 23 had negative salivary viral levels hence they were excluded from further analysis due to effect on overall results findings. Despite removal of those who tested negative at the analysis stage, age and gender was still present with even distribution.

4.4.1 SARS-COV-2 VIRAL LOAD BY MOUTHWASH AT BASELINE

The general characteristics of the baseline values (T0) are shown in Table 11. The highest Ct value at baseline (T0) was 22.64 for Placebo followed by 20.93 for CHX and lastly 20.85 for PI. Indicating that Placebo group had the lowest mean viral load.

Table 11: SARS-CoV-2 viral load with each Mouthwash at Baseline (n = 69)

Mouthwash agent	Total (n)	Baseline (T0) Ct Values
Povidone- Iodine (PI)	25	20.85
Chlorhexidine (CHX)	21	20.93
Placebo (distilled water)	23	22.64

4.4.2 SARS-COV-2 VIRAL LOAD BY AGE AT BASELINE

By age the highest Ct value was in the 18 – 30 age group at 22.23, followed by > 45 age group at 21.10 and the lowest for the 31 – 45 age group at 20.79. This Indicated that the 31 – 45 age group had the highest mean viral load level. An analysis of variance (ANOVA) test showed a non-statistically significant difference in SARS CoV-2 viral load at baseline (T0) by age groups, ($F(2, 66) = 0.896$, $p = .413$). This infers that the differences in SARS CoV-2 viral load at baseline (T0) were not significantly dependent on the participants' age groups ($p= 0.413$) (table 12)

Table 12: Comparison of SARS CoV-2 viral load by age groups at Baseline (T0) (n = 69).

				95% CI for Mean				
Age (years)	n (%)	M	SD	Lower	Upper	df	F	p
18-30	30(43.5)	22.2	3.9	20.8	23.7	2, 66	0.896	.413
31-45	27(39.1)	20.8	4.4	19.1	22.5			
> 45	12(17.4)	21.1	4.3	18.4	23.8			

Analysis of variance (ANOVA) test was applied.
CI; Confidence Interval.

4.4.3 SARS-COV-2 VIRAL LOAD BY GENDER AT BASELINE

By gender, males had lower Ct values at 20.0 than the females at 22.7 indicating that the males had a higher mean viral load level. A one sample t-test showed a statistically significant difference in SARS CoV-2 viral load at baseline (T0) by gender ($t(67) = 2.833$, $p < 0.05$). This infers that the differences in SARS CoV-2 viral load at baseline (T0) was significantly dependent on the participants' gender ($p = .006$) (table 13).

Table 13: Comparison of SARS CoV-2 viral load by gender at Baseline (T0) (n = 69).

				95% CI for Mean				
Gender	*n (%)*	*M*	*SD*	*Lower*	*Upper*	*df*	*t*	*p*
Male	31(44.9)	20.0	4.0	-4.6	-0.8	67	-2.833	.006
Female	38(55.1)	22.7	3.9					

t test was applied.
CI; Confidence Interval.

4.5 VIRAL LOAD LEVELS 30 MINUTES AFTER INTERVENTION

4.5.1 SARS-COV-2 VIRAL LOAD BY MOUTHWASH AT 30 MINUTES INTERVAL

The general characteristics of the viral load values at the 30 minute interval are shown in Table 14. The highest Ct values after 30 min intervention with a mouthwash was seen for PI at 23.2 followed by 23.1 for the distilled water and the lowest was 22.5 for CHX. Indicating that CHX had the highest mean viral load.

Table 14: SARS-CoV-2 viral load with each Mouthwash at 30 minutes interval (T1) (n = 69)

Mouthwash agent	Total (n)	30 min Intervention (T1) Ct Value
Povidone- Iodine (PI)	25	23.2
Chlorhexidine (CHX)	21	22.5
Distilled water (Placebo)	23	23.1

4.5.2 SARS-COV-2 VIRAL LOAD BY AGE AT 30 MINUTES INTERVAL

By age the highest Ct value at 30 min interval after the intervention was seen with the 18 – 30 age group at 23.4, followed by > 45 at 23.2 and the lowest the 31 – 45 age group at 22.5. Indicating that the 31-45 age group had the highest mean viral load level after intervention. An analysis of variance (ANOVA) test did not show statistically significant difference in SARS CoV-2 viral load at 30 minutes interval (T1) by age groups, ($F(2, 66) = 0.379$, $p = .686$). This infers that the differences in SARS CoV-2 viral load at 30 minutes interval (T1) were not significantly dependent on the participants' age groups ($p= .686$) (table 15).

Table 15 : Comparison of SARS CoV-2 viral load by age groups at 30 minutes interval (T1) (n = 69).

				95% CI for Mean				
(years)	n (%)	M	SD	Lower	Upper	df	F	p
18-30	30(43.5)	23.4	3.7	22.0	24.7	2, 66	0.379	.686
31-45	27(39.1)	22.5	4.2	20.8	24.1			
> 45	12(17.4)	23.2	4.0	20.7	25.7			

Analysis of variance (ANOVA) test was applied.
CI; Confidence Interval.

4.5.3 SARS-COV-2 VIRAL LOAD BY GENDER AT 30 MINUTES INTERVAL

By gender, females were shown to have a higher Ct value at 24.1 while the males had a lower value at 21.6 indicating that the males had higher viral load levels. A one sample t-test showed a statistically significant difference in SARS CoV-2 viral load at 30 minutes interval (T1) by gender, ($t(67) = 2.842$, $p < 0.05$). This infers that the differences in SARS CoV-2 viral load at 30 minutes interval (T1) was significantly dependent on the participants' gender (p= .006) (table 16).

Table 16: Comparison of SARS CoV-2 viral load by gender at 30 minutes interval (T1) (n = 69).

				95% CI for Mean				
Gender	n (%)	M	SD	Lower	Upper	df	t	p
Male	31(44.9)	21.6	4.1	-4.4	-0.8	67	-2.842	.006
Female	38(55.1)	24.1	3.4					

t test was applied.
CI; Confidence Interval.

4.6 EFFECT OF MOUTHWASH ADMINISTRATION ON SARS-COV-2 VIRAL LOADS.

4.6.1 PERCENTAGE CHANGES IN SARS-COV-2 VIRAL LOAD WITH EACH MOUTHWASH AT BASELINE AND AFTER 30 MIN INTERVENTION

Baseline and after 30 min intervention showed that Povidone-Iodine (PI) had the largest change in viral load at 2.4 (11.4%) followed by Chlorhexidine (CHX) at 1.6 (7.7%) with the least change in viral load observed for the distilled water at 0.5 (2.2%) (Table 17). Differences between PI and distilled water was statistically significant ($F=9.361$, $p<0.001$), and the difference between CHX and distilled water was statically significant ($F=8.962$, $p<0.021$).

Table 17 : Percentage changes in SARS-CoV-2 viral load with each Mouthwash at Baseline and after 30 min intervention (n = 69)

	Mean Viral load (ct)		**Change in viral load**	
Mouthwash agent	Baseline (T0)	30 min. Interval (T1)	Ct	% change
Povidone- Iodine (PI)	20.9	23.2	2.4	11.4
Chlorhexidine (CHX)	20.9	22.5	1.6	7.7
Distilled water (Placebo)	22.6	23.1	0.5	2.2

Figure 1 shows that there was an increase in the Ct values from T0 to T1 thereby indicating a reduction in viral load levels after intervention with PI and CHX.

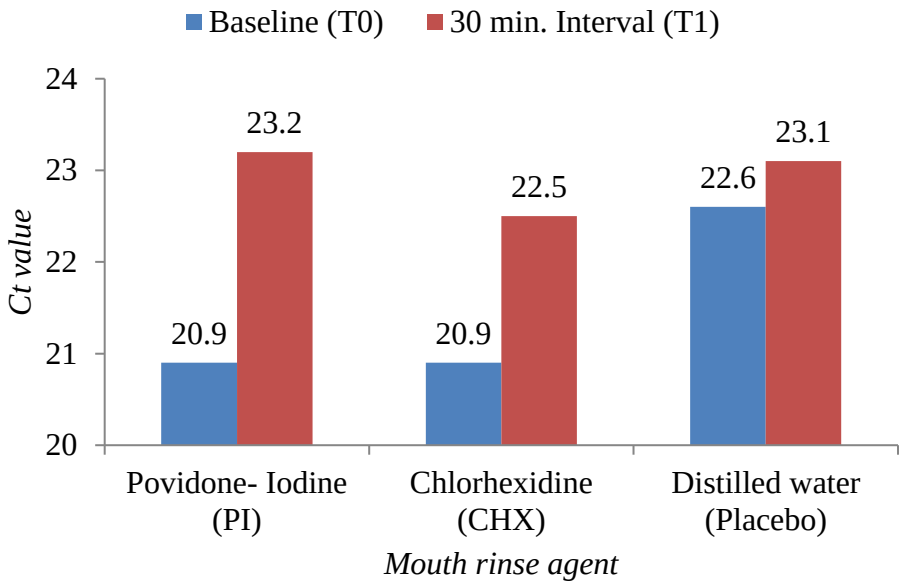

Figure 2: SARS-CoV-2 viral loads for mouth rinse agents at baseline (T0) and 30 minutes intervals (T1).

4.6.2 COMPARISON BETWEEN CHANGES IN SARS-COV-2 VIRAL LOAD BETWEEN INTERVENTION AND CONTROL GROUPS

An analysis of covariance (ANCOVA) test was applied to determine the changes in SARS-CoV-2 viral load between intervention and control groups for the mouthwash agents. The estimated means adjusted for the covariate showed that Povidone-Iodine (PI) had the largest mean of 23.2, followed by distilled water with a mean of 23.1 and Chlorhexidine (CHX) with a mean of 22.5. The ANCOVA test showed a statistically significant overall difference in viral loads between the intervention and control groups for the mouthwash agents with a statistically significant difference between Povidone-Iodine (PI) and distilled water (Placebo) ($F=7.635$, $p=0.001$). This infers that the mouthwash agents had a statistically significant effect on the participants' SARS-CoV-2 viral loads post intervention (table 18).

Table 18:. Comparison between changes in SARS-CoV-2 viral load between intervention and control groups for the mouthwash agents (n = 69).

Mouthwash agent		Viral load		95% CI			
		M Dif.	p	lower	Upper	F	P (ANCOVA)
PI (1%)	CHX (0.2%)	0.8	.272	-0.3	1.8	7.635	.001
	Distilled Water	1.7	.001	0.6	2.8		
CHX (0.2%)	PI (1%)	-0.8	.272	-1.8	0.3		
	Distilled Water	0.9	.122	-0.2	2.1		

Analysis of covariance (ANCOVA) test was applied.

M Dif.; Mean difference.

CI; Confidence Interval.

4.6.3 COMPARISON BETWEEN CHANGES IN SARS-COV-2 VIRAL LOAD BETWEEN INTERVENTION AND CONTROL GROUPS BY AGE

Comparison of Baseline and after 30 min intervention showed that > 45 age group had the largest change percentage in viral load at 10.0% followed by 31 – 45 at 8.2% and lastly 18 – 30 age group at 5.4%. (Table 19).

An analysis of variance (ANOVA) test did not find any statically significant difference in viral load change by age groups (F (2,66) =1.558, p= 0.218).

Table 19: Percentage changes in SARS-CoV-2 viral load with each age group at Baseline and after 30 min intervention (n = 69)

	Ct		Change in viral load	
Age (years)	Baseline (T0)	30 min. Interval (T1)	Ct	% Change
18-30	22.2	23.4	1.2	5.4
31-45	20.8	22.5	1.7	8.2
> 45	21.1	23.2	2.1	10.0

Figure 2 shows that there was an increase in the Ct values from T0 to T1 thereby indicating a reduction in viral load levels after intervention with the mouthwashes in all age groups.

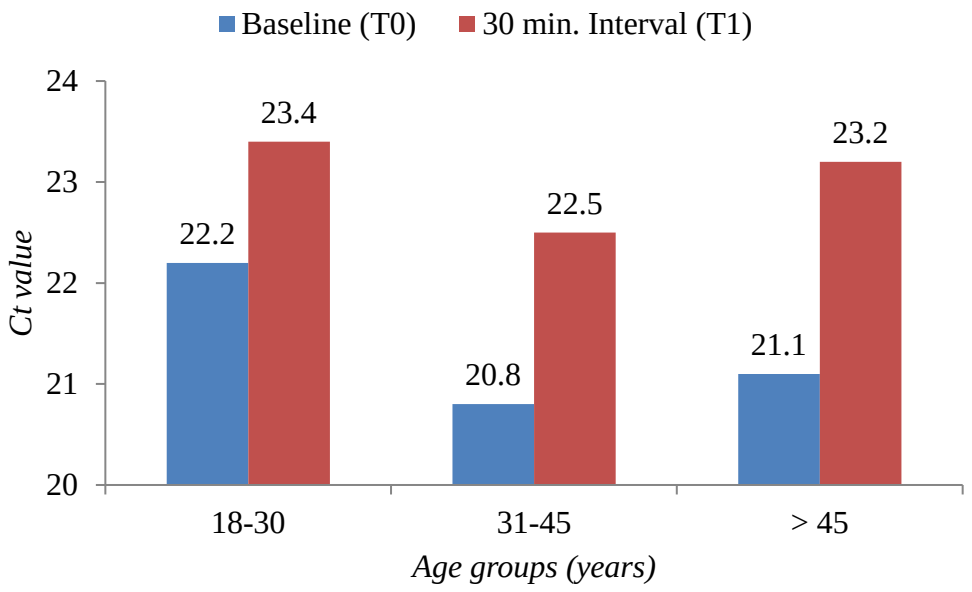

Figure 3: SARS-CoV-2 viral loads for age groups at baseline (T0) and 30 minutes intervals (T1)

4.6.4 COMPARISON BETWEEN CHANGES IN SARS-COV-2 VIRAL LOAD BETWEEN INTERVENTION AND CONTROL GROUPS BY GENDER

Comparison of Baseline and after 30 min intervention showed that males had the larger percentage change in Ct values at 8.0% compared to females at 6.2% (Table 20).

A one sample t-test showed a non-statically significant difference in viral load change by gender, (t (67)=0.367, p= 0.714).

Table 20: Percentage changes in SARS-CoV-2 viral load with each gender at Baseline and after 30 min intervention (n = 69)

	Ct Value		Change in viral load	
Gender	Baseline (T0)	30 min. Interval (T1)	Ct	% Change
Male	20.0	21.6	1.6	8.0
Female	22.7	24.1	1.4	6.2

Figure 3 shows that there was an increase in the Ct values from T0 to T1 thereby indicating a reduction in viral load levels after intervention for both males and females.

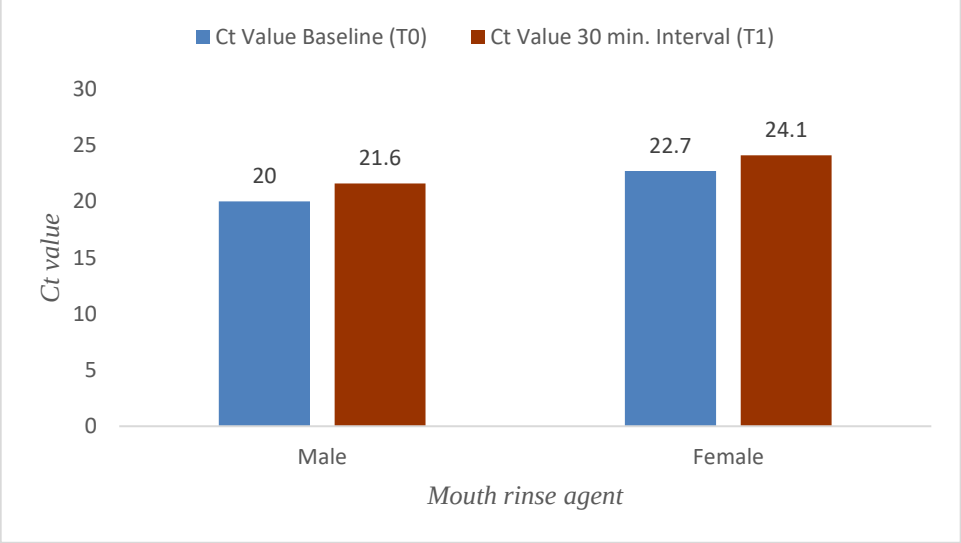

Figure 4: SARS-CoV-2 viral loads for gender groups at baseline (T0) and 30 minutes intervals (T1)

CHAPTER 5

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 DISCUSSION

Research has been ongoing worldwide, addressing infection control protocols, but little attention has been paid to the efficacy of mouthwashes particularly regarding aerosol reduction in clinical setting during the COVID -19 viral crisis. This study aimed at enabling health bodies to enhance "infection control" guidelines for dental practitioners.

This clinical study examined the efficacy of mouth-rinses on SARS-CoV-2 viral loads in COVID-19 patients. This study provided an insight into the viral quantity levels in saliva, which is significant due to the amounts of aerosols generated during dental procedures[54]. The Ministry of Health (MOH) in Kenya has recommended the use of pre-procedural mouth-rinses during dental treatment; therefore, this present study will provide much needed evidence on the efficacy of mouthwashes for salivary viral load reduction in a cohort of COVID-19 patients in Kenya.

This study has analyzed the in vivo effect of two mouthwashes versus distilled water (placebo), on the salivary SARS CoV-2 viral load (viral particles per mL of saliva). This is the first time to our knowledge that SARS-CoV-2 infectivity after an antiseptic mouth rinse has been studied within the Kenyan population. Specifically, the effect of two different mouthwashes, including PI (Povidone Iodine) 1% and CHX (Chlorhexidine) 0. 2% and distilled water as the placebo group, were analyzed. Saliva was reaffirmed as a valid substrate for the study of SARS CoV-2 viral load with a 75% positivity rate, which was similar to results found by Biber et al with a 79% detection rate from their saliva samples[55]. Amongst the 92 enrolled and randomized patients, 23 had negative salivary viral loads. These were excluded from analysis due to possible effect on overall results findings.

5.1.1 SOCIODEMOGRAPHIC CHARACTERISTICS

The participants age range was 18 -72 years with a mean of 34 years and a broad standard deviation of 12.8 years (Table 3). This implied the diverse and varied ages of adults that were hospitalized for COVID-19 treatment at the study area. According to the current study, more participants were of female gender in comparison with males (Table 4). The higher ratio of adult females to males being hospitalized indicated that females generally had better health-seeking behavior and agreed with the findings by Thompson et al 2013[56]. Thompson et al found in a Canadian population woman reporting they visited their primary care provider to a greater extent than did men[56].

Thirty three percent (33%) of the participants had achieved primary level education, 37% secondary education while only 22% had completed tertiary education (Table 5). There was no statistically significant disparity regarding level of education and is possibly because this study was conducted in an urban setting whereby, the urban population is perhaps more educated.

Most participants were found to be residing in Nairobi. This was so because the study was carried out in Hospitals within Nairobi. Hence most participants were likely to have been drawn from the city and its close environs.

5.1.2 VACCINATION STATUS

Globally, vaccinations against COVID-19 have been found to be one of the most effective ways to control SARS CoV-2 transmission and morbidity. It is recommended that one gets vaccinated with all doses as well as booster doses of COVID-19 vaccines to protect yourself and help prevent new variants from emerging. This study found that 57.6% (53) of the participants were not vaccinated while 42.2%(39) were vaccinated. This is concurrence with the Ministry of Health Vaccination Status in Kenya report that showed as of December 2022 only 23 million (18%) doses of vaccines have been administered in a current population of 53 million [57]. In this study AstraZeneca was the most common vaccine at 46.2%, followed by Moderna at 30.8% and Pfizer at 17.9% with Johnson n Johnson being

the least administered vaccine at 5.1%. Globally the most administered vaccine type is AstraZeneca followed by Pfizer, Moderna and followed by Johnson n Johnson. In this study AstraZeneca could have been the most widely used because of the availability as it was sourced and distributed by the Government of Kenya and thus was readily available, accessible and free of charge rather than relying only on the global trend. Of the vaccinated participants, majority 66.7% had received 1 dose of vaccination while 33.3% had 2 doses of vaccinations administered (Appendix 7).

This study observed that none of the vaccinated participants had taken a booster vaccination. The MOH status report also showed that only 1.7 million (3%) of the total population within Kenya had received booster doses[57]. I speculate that the resistance to the booster doses was possibly due to lack of awareness and knowledge about the importance of booster vaccines within the Kenyan population. No statistical significance was observed between the vaccination status and type in relation to the to the age thus concluding, that age had no influence on whether an individual was vaccinated or not.

Whereas there was a statistical significance ($\chi 2 = 4.319$, $p=0.038$) between the vaccination status and gender showing that 26% of the males had been vaccinated while only 15% of females were found to have been be vaccinated (Table 7). This is in accordance with a meta-analysis that found lower vaccination intentions amongst women compared to men following a review between 66 studies[58]. Males being more willing to be vaccinated has already been reported in a study within a Caucasian population [59] although, contrasting results showing a higher intention to be vaccinated among women have also been described [60]. A survey conducted in 10 countries investigated the gender disparity of vaccinations and suggests that females hesitate to get vaccinated partly due to skepticism, since women are less likely to believe that vaccination is the only solution to COVID-19 and more likely to believe that COVID-19 was created by large corporations[61].

No statistically significant association was seen between the various vaccination characteristics and participants' education levels (Table 7). However, limited health literacy is prevalent and is consistently associated with levels of education as seen in other studies. Nevertheless, it should be argued that location of study, economic status, social

beliefs, and religious beliefs are numerous factors that influence the decision to be vaccinated[62].

5.1.3 SARS COV-2 VIRAL LOAD

It is noteworthy to observe that SARS-CoV-2 viral load was detected in 75%(n=69) of the saliva samples. This finding suggests that SARS-CoV-2 might be secreted from the salivary glands rather than the nasopharynx [63]. Thus, from results of this study, saliva specimens can be used for the diagnosis of SARS-CoV-2 infection [64]. Aerosol generation of the virus is relevant as transmission of coronaviruses occurs primarily by close contact via respiratory droplets, which are generated by sneezing, coughing, breathing, and talking[65].

Before the mouthwash, the range of mean Ct values was between 18.4 to 23.8 at T0 (baseline samples) in comparison to age (Table 12). There was no statistically significant correlation in reference to the SARS CoV-2 viral loads and age at baseline ($F(2, 66) = 0.896$, $p = .413$). After the 30 min interval with the mouthwash the range of mean Ct values was between 20.7 to 25.7 at T1 (30 mins interval) in comparison to age (Table 15). There was no statistically significant correlation in reference to the SARS CoV-2 viral loads and age at 30 minutes interval (T1) ($F(2, 66) = 0.379$, $p = .686$).

These results represent an interesting perspective as there is conflicting evidence present in literature regarding this. A cohort study conducted amongst 270 thousand participants in the Netherlands showed an increasing SARS CoV-2 viral load with an increase in age, whereas another study in the United States involving 4 thousand participants showed no viral load differences in various age brackets[66,67].

By gender this study found before the mouthwash intervention females were shown to have a higher mean Ct value at 22.7 while the males had a mean Ct value at 20.0 at baseline (Table 13). There was a statistical significance seen in reference to the SARS CoV-2 viral loads by gender at baseline ($F(2, 66) = 0.379$, $p = .686$) (Table 13). While also after the 30 min interval females were shown to have a higher mean Ct value at 24.1 while the males

had a lower mean Ct value at 21.6 (Table 19). There was also a statistical significance seen in reference to the SARS CoV-2 viral loads by gender after a 30 min Interval for both genders ($t(67) = 2.842, p < 0.05$) (Table 16). This therefore indicated that before and after the intervention males had higher mean viral loads compared to females. This could be due to a difference in immune response in which females develop a higher immune response to infectious agents, making them less susceptible to disease [68].

A contradictory study conducted by Mahallawi et al showed females had higher SARS CoV-2 viral loads in relation to males[69]. However, evidence in results worldwide is controversial as a comparative study by Jacot et al and Kleiboeker et al found comparable viral loads between both males and females [70,71].

Interestingly mean Ct values for the three mouthwashes at baseline(T0) were (PI- 20.85, CHX- 20.93, distilled water- 22.64) and the mean after 30 min intervention (T1) was (PI- 23.2, CHX- 22.5, Distilled water- 23.1) This showed an increase in Ct values therefore a subsequent reduction in Viral loads (Table 16).

PI and CHX mouthwashes were effective in reducing the SARS-CoV-2 viral load in the saliva for a short-term period of 30 minutes in comparison to the distilled water. The interventions were successful as presented by the mean differences of 0.5 (2.2%) for the distilled water, 1.6 (7.7%) for CHX and 2.4 (11.4%) for PI, showing greater viral load reductions after using the mouthwashes (Table 17). No Superiority was seen between the two interventions PI and CHX as seen by the p value of 0.001 (Table 18). However, the effect of PI was greater ($p<0.001$) as seen in (Table18). The proportion depletion of SARS CoV-2 viral load was significantly greater in PI at 11.4% than compared to CHX at 7.7%. The highest mean difference was seen with the PI mouthwash and the lowest in the distilled water. Thus, it is inferred that PI and CHX as a mouth rinse may have significant effects in suppression of salivary viral load thereby reducing the risk of SARS CoV-2 in clinical setting.

These results are consistent with those of Anderson et al who concluded that a 30 second contact with PI yields a high virucidal activity reduction[72]. On the other hand, Ferrer et al evaluated the use of 2% PI on the reduction of salivary viral load and found no statistically

significant changes in salivary viral load after the use of the different mouthwashes, including PI [73].

Similarly, Costa et al found that 0.12% CHX decreased the viral load after 60 minutes[74]. This study showed similar results in comparison to these other studies however, these previous clinical trials involved small sample sizes and had no control groups. In contrast, no effect was found in 6 patients after using 15 ml of chlorhexidine for 30 s in a randomized clinical trial by Seneviratne et al[45]. Reasons for the divergent results might be the higher volume of salivary sample collected (5ml) and the longer time interval between collections (5 min, 3 h and 6 h) and a relatively small sample size. Considering that chlorhexidine compounds are effective against lipid-enveloped viruses, the participants were administered a chlorhexidine (0.2%, 15 mL) mouthwash and found viral suppression for 30minutes after using the mouthwash once.

In the present study, the salivary viral load was assessed 30 min after using the interventions because this was the time required to operationalize and standardize the saliva collection time and was used in previous studies, this time is also adequate to carry out most dental procedures. [75].

5.2 CONCLUSION

Based on the findings of this study, the following was concluded;

i. PI mouthwash was effective in reducing SARS-CoV-2 salivary viral load.
ii. CHX mouthwash also reduced SARS-CoV-2 salivary viral load in comparison to the placebo.

5.3 RECOMMENDATIONS

Based on the findings of this study, the following was recommendations are made;

i. PI can be used as a pre-procedural mouthwash as well as an adjunct to PPE to help reduce the salivary load of SARS CoV-2 in healthcare services where saliva exposure is expected, such as dental practices as well as in situations involving close contact between people in domestic and public spaces.
ii. The results achieved suggest that Povidone Iodine (PI) can be useful in making of Mouthwash policy and protocol for COVID-19 treatment in health care settings.
iii. There is need for further studies to be done on PI at different concentrations and time intervals to determine their exact efficacy in SARS-CoV-2

5.4 LIMITATIONS

Lack of clinical data and the consequent inability to correlate laboratory values with illness stage or severity are a limitation in this study. Additionally, several samples at different time intervals were not taken in this study therefore, it was not possible to determine the survival of the virus over time.

A drawback of this study is that the RT-PCR assay measures the relative differences in mRNA template abundance and not viral infectivity or viability. Nevertheless, given the limitations in culturing SARS-CoV-2, viral RNA load has been considered a reliable substitute marker[76].

The study was also hospital based, posing a challenge in making inferences to the general population.

The reagents and materials required were costly with the investigator bearing the costs.

5.5 CONFLICT OF INTEREST AND SOURCE OF FUNDING STATEMENT

The cost of the study was undertaken solely by the principal investigator for scientific and academic purposes. There was no conflict of interest related to this study.

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Appendix

Appendix 1: Ethical Approval

UNIVERSITY OF NAIROBI
FACULTY OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
Tel:(254-020) 2726300 Ext 44355

KNH-UON ERC
Email: uonknh_erc@uonbi.ac.ke
Website: http://www.erc.uonbi.ac.ke
Facebook: https://www.facebook.com/uonknh.erc
Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/202

25th May, 2022

Dr. Parina Bhupendra Patel
Reg. No. V60/34561/2019
Periodontology/Community and Preventive Dentistry Unit
Dept. of Dental Sciences
Faculty of Health Sciences
University of Nairobi

KENYATTA NATIONAL HOSPITAL APPROVED 25 MAY 2022 P.O. Box 20723 – 00202 NAIROBI KNH/UoN-ERC

Dear Dr. Patel,

RESEARCH PROPOSAL: INFLUENCE OF POVIDONE IODINE AND CHLORHEXIDINE ON THE SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS-2 VIRAL LOAD USING A SALIVA TEST AMONG PATIENTS HOSPITALIZED WITH CORONAL VIRUS DISEASE-19 (P30/01/2022)

This is to inform you that KNH-UoN ERC has reviewed and approved your above research proposal. Your application approval number is **P30/01/2022.** The approval period is 25th May 2022– 24th May 2023.

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used.
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by KNH-UoN ERC.
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to KNH-UoN ERC 72 hours of notification.
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH-UoN ERC within 72 hours.
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to KNH-UoN ERC.

Appendix 2: Permission Letter

UNIVERSITY OF NAIROBI
FACULTY OF HEALTH SCIENCES
DEPARTMENT OF DENTAL SCIENCES
UNIT OF PERIODONTOLOGY/COMMUNITY & PREVENTIVE DENTISTRY

FROM: Unit Head,
Periodontology/Community & Preventive Dentistry

DATE: March 3, 2022

TO: To whom it may concern

REF: UON/FHS/DS/1/7

Re: Dr Parina Patel Research

Dr Parina is a third year Master of Dental Surgery in Periodontology student in the department of Dental Surgery of the University of Nairobi.

She is now in the process of collecting data for her thesis entitled **"Influence of Povidine Iodine and chlorhexidine on Severe Acute Respiratory Syndrome Corona-2 viral load".**

The study design requires that she collects saliva from patients hospitalized with COVID-19,and of whom povidine iodine/chlorhexidine mouth wash has been administered.

Your health facility was identified as a suitable one for the study since it accommodates COVID-19 patients. As such I write to request you to accord her any assistance she may require with respect to this study while at your health facility.

The results thereof are expected to shade more light in the management of patients with COVID-19, especially dental patients.

Thank you.

Dr Bernard N Mua

Head, Periodontology, Community and Preventive Dentistry

Appendix 3: Consent Form English Version

UNIVERSITY OF NAIROBI (UoN) COLLEGE OF HEALTH SCIENCES
P O BOX 19676 code 00202
Telegrams: varsity
(254-020) 2726300
Ext 44355
KNH-UoN ERC

Email: uonknh_erc@uonbi.ac.ke
Website: http://www.erc.uonbi.ac.ke
Twitter: @UONKNH_ERC
ttps://twitter.com/UONKNH_ERC

KENYATTA NATIONAL HOSPITAL (KNH)
P O BOX 20723
Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

PARTICIPANT INFORMATION AND CONSENT FORM

SAMPLE ADULT CONSENT
FOR ENROLLMENT IN THE STUDY

Title of Study: Influence of Povidone Iodine and Chlorhexidine on the Severe Respiratory Syndrome Coronavirus-2 Viral load using a saliva test among hospitalized with the Corona Virus disease-19

Principal Investigator\and institutional affiliation: Dr. Parina Patel University of Nairobi

Co-Investigators and institutional affiliation: N/A

Introduction:

I would like to tell you about a study being conducted by the above listed researcher. The purpose of this consent form is to give you the information you will need to help you decide whether to be a participant in the study. Feel free to ask any questions about the purpose of the research, what happens if you participate in the study, the possible risks and benefits,

your rights as a volunteer, and anything else about the research or this form that is not clear. When we have answered all your questions to your satisfaction, you may decide to be in the study or not. This process is called 'informed consent'. Once you understand and agree to be in the study, I will request you to sign your name on this form. You should understand the general principles which apply to all participants in medical research:

i) Your decision to participate is entirely voluntary

ii) You may withdraw from the study at any time without necessarily giving a reason for your withdrawal

iii) Refusal to participate in the research will not affect the services you are entitled to in this health facility or other facilities. We will give you a copy of this form for your records.

May I continue? YES / NO

This study has approval by The Kenyatta National Hospital-University of Nairobi Ethics and Research Committee Protocol No. _____

What is this study about?

The study is aimed at establishing the efficacy of mouthwashes on SARS-CoV-2 salivary viral loads. The information I get is part of my research for a thesis as a partial fulfillment for the degree of Master of Dental Surgery in Periodontology.

How do you participate?

I shall ask you some questions on the knowledge and practices of your oral health. I will get a sample of your saliva and ask you to rinse with a mouth wash. The examinations shall be carried out using clean (sterile) instruments and no invasive procedures shall be performed.

WHAT WILL HAPPEN IF YOU DECIDE TO BE IN THIS RESEARCH STUDY?

If you agree to participate in this study, the following things will happen:

You will be interviewed by a trained interviewer in a where you feel comfortable answering questions. The interview will last approximately 5 minutes.

After the interview has finished you will be asked to collect saliva in the mouth and spit saliva in a sterile container**.**

If you agree to provide your contact information, it will be used only by people working for this study and will never be shared with others. The reason why we may need to contact you is in the unlikely event some biodata is lost.

ARE THERE ANY RISKS, HARMS DISCOMFORTS ASSOCIATED WITH THIS STUDY?

Medical research has the potential to introduce psychological, social, emotional and physical risks. Effort should always be put in place to minimize the risks. One potential risk of being in the study is loss of privacy. We will keep everything you tell us as confidential as possible. We will use a code number to identify you in a password-protected computer database and will keep all our paper records in a locked file cabinet. However, no system of protecting your confidentiality can be secure, so it is still possible that someone could find out you were in this study and could find out information about you.
Also, answering questions in the interview may be uncomfortable for you. If there are any questions you do not want to answer, you can skip them. You have the right to refuse the interview, or any questions asked during the interview.
It may be embarrassing for you to have oral examination. We will do everything we can to ensure that this is done in private. Furthermore, all study staff and interviewers are professionals with special training in these examinations/interviews.
You may feel some discomfort when doing intra oral examination. In case of an injury, illness or complications related to this study, contact the study staff right away at the number provided at the end of this document. The study staff will treat you for minor conditions or refer you when necessary.

WHAT IF YOU HAVE QUESTIONS IN FUTURE?

If you have further questions or concerns about participating in this study, please call or send a text message to the study staff at the number provided at the bottom of this page.

For more information about your rights as a research participant you may contact the Secretary/Chairperson, Kenyatta National Hospital-University of Nairobi Ethics and Research Committee Telephone No. 2726300 Ext. 44102 email uonknh_erc@uonbi.ac.ke. The study staff will pay you back for your charges to these numbers if the call is for study-related communication.

WHAT ARE YOUR OTHER CHOICES?

Your decision to participate in research is voluntary. You are free to decline participation in the study and you can withdraw from the study at any time without injustice or loss of any benefits.

CONSENT FORM (STATEMENT OF CONSENT)

Participant's statement

I have read this consent form or had the information read to me. I have had the chance to discuss this research study with a study counselor. I have had my questions answered in a language that I understand. The risks and benefits have been explained to me. I understand that my participation in this study is voluntary and that I may choose to withdraw any time. I freely agree to participate in this research study.

I understand that all efforts will be made to keep information regarding my personal identity confidential.

By signing this consent form, I have not given up any of the legal rights that I have as a participant in a research study.

I agree to participate in this research study:	**Yes**	**No**
I agree to have saliva preserved for later study:	Yes	No
I agree to provide contact information for follow-up:	Yes	No

Participant Serial number:

_____

Participant signature / Thumb stamp _____ Date _____

Witness signature _____ Date _____

Researcher's statement

I, the undersigned, have fully explained the relevant details of this research study to the participant named above and believe that the participant has understood and has willingly and freely given his/her consent.

Researcher 's Name: Dr.Parina Patel **Date**: _____

Signature

_____

Role in the study: Principal investigator

For more information contact

The Principal Investigator

Dr. Parina Patel

School of Dental Sciences, University of Nairobi,

Tel: 0708905358

Lead Supervisor

Dr. Wetende Andrew

Unit Head- Head of Periodontology

Unit of Periodontology/Community and Preventive Dentistry, Department of Dental Sciences, Faculty of Health Sciences, University of Nairobi

The Secretary/Chairperson,

Kenyatta National Hospital-University of Nairobi Ethics and Research Committee

Telephone No. (254-020) 2726300-9

Email: uonknh_erc@uonbi.ac.ke.

Appendix 4: Consent Form Swahili Version

FOMU YA RIDHAA

SAMPULI YA RIDHAA YA MTU MZIMA

YA USAJILI WA UTAFITI

Mada ya utafiti: Ufanisi wa Povidone Iodini na Chlorhexidine kwenye mzigo wa virusi wa SARS-COV-2 kwa kutumia vipimo vya mate katika wagonjwa waliolazwa wa COVID-19.

Mkuu wa uchunguzi na uhusiano wa taasisi: Daktari Parina Patel
Chuo kikuu cha Nairobi
Wachunguzi wenza na uhusiano wa taasisi: Haihusiki

Utangulizi:

Ningetaka kukueleza kuhusu utafiti unaofanywa na watafiti ambao wametajwa hapo juu. Lengo la fomu hii ya ridhaa ni kukuwezesha kufanya uamuzi wa iwapo utashiriki katika utafiti au la. Kuwa mwepesi wa kuuliza swali lolote kuhusiana na lengo la utafiti,nini hufanyika iwapo utashirikio kwenye utafiti, hatari na manufaa ya utafiti, haki yako kama mtu aliyejitolea kwa hiari na jambo jingine lolote kuhusiana na utafiti au fomu hii ambalo halijaeleweka. Baada ya kuyajibu maswali yako vilivyo, waweza kuamua kushiriki kwenye utafiti au kutoshiriki. Mchakato huu unafahamika kama ‘ridhaa inayofahamika’. Pindi tu utakapoelewa na kukubali kuwa kwenye utafiti, nitaomba ulinakili jina lako na kutia sahihi kwenye fomu hii. Yafaa uelewe sharia za kawaida ambazo hutumiwa na washiriki wote katika utafiti wa kimatibabu:

i) Uamuzi wako wa kushiriki ni wa hiari kabisa
ii) Waweza kujiondoa kwenye utafiti wakati wowote bila kupatiana sababu ya kufanya hivyo.

iii) Kukataa kushirikio kwenye utafiti hakutaathiri wajibu uanaopaswa kutekeleza katika kituo hiki cha afya ama vituo vinginevyo. Tutakupa nakala ya fomu hii kwa ajili ya rekodi zako

Naweza kuendelea? NDIO / LA

Utafiti huu umeidhinishwa na hospitali ya Kitaifa ya Kenyatta-Kamati ya maadili na utafiti Chuo Kikuu Cha Nairobi, Nambari ya itifaki. _____

Utafiti huu unahusu nini?

Utafiti huo unalenga kubainisha ufanisi wa waosha vinywa kwenye viwango vya virusi vya SARS-CoV-2. Maelezo ninayopata ni sehemu ya utafiti wangu wa nadharia kama utimilifu wa sehemu ya shahada ya Upasuaji wa Meno katika Periodontology.

Nitashiriki vipi?

Nitakuuliza maswali kuhusiana na unayofahamu kwenye afya ya kinywa. Nitapata sampuli za mate yako na kukuuliza suuza kwa kuosha kinywa.

Uchunguzi utafanywa kwa kutumia vifaa safi na hakuna shurutisho litakalofanywa.

NI NINI KITAKACHOFANYIKA IWAPO UTAAMUA KUWEKO KWENYE UTAFITI?

Iwapo utakubali kushiriki kwenye utafiti, mambo yafuatayo yatafanyika:

Utahojiwa na mtu ambaye amepitia mafunzo ambapo utaweza kuyajibu maswali. Mahojiano hayo yatachukuwa yapata muda wa dakika tano. Mahojiano hayo yatahusisha mada kama vile usafi kinywani na ufahamu wa usafi kinywani na jinsi ya kufanya usafi huo. Mahojiano yalikamilika utaulizwa ukusanye mate kwa dakika tano kuoka kinywani na kuyatia katika chombo safi.

Ukikubali kutupa nambari ya simu itatumiwa tu na watafiti katika utafiti huu na kamwe haitapewa mtu mwingine yeyote.Sababu yetu kuchukua nambari yako ya simu ni ili tuweze kuwasiliana nawe iwapo data itapotea.

JE, KUNA HATARI ZOZOTE AU MADHARA YANAYOHUSISHWA NA UTAFITI HUU?

Utafiti wa kimatibabu una uwezo wa kusababisha hatari za kisaikolojia, katika mahusiano, hisia na kimwili.Yafaa tujaribu tuwezavyo kupunguza hatari hizo.Hatari moja ambayo yaweza kutokea ni ukosefu wa siri.Yote utakayotuambia yatabaki kuwa siri.Tutatumia kodi fulani kukutambua katika tarakilishi iliyo na neon la siri. Data na nakala zetu zote tutazifungia kwa kabati. Hata hivyo,hakuna chombo cha kuhifadhi siri yako ambacho ni salama kabisa na huenda mtu akafumbua kwamba ulishiriki katika utafiti na apate habari kukuhusu.

Aidhaa kujibu maswali kwenye mahojiano huenda kukawa kugumu kwako.Iwapo kuna maswali hutaki kujibu waweza kuyaacha.Una haki ya kukataa mahojiano au swali lolote litakaloulizwa kwenye mahojiano.

Inawezekana liwe ni jambo la aibu kwako kufanyiwa uchunguzi.Tutahakikisha ya kwamba yote hayo yatafanyiwa mahali pa siri.Hali kadhalika watakaofanya mahojiano ni watu wenye weledi na ujuzi. Huenda usihisi vizuri wakati wa kukaguliwa kinywani. Pakitokea ya kwamba umejeruhiwa , umekuwa mgonjwa au shida nyingine inayohusiana na utafiti huu imetokea piga nambari utakayoona mwishoni mwa nakala hii haraka iwezekanavyo.Wahudumu watakutibu magonjwa madogo madogo au wakutume kwingineko iwapo itahitajika kufanya hivyo

IWAPO UKUMBANE NA MASWALI SIKU ZA USONI

Iwapo utakuwa na maswali Zaidi kuhusu utafiti huu tafadhali piga simu au utume arafa kwa nambari iliyoko mwishoni mwa nakala hii ili kuwasiliana na wahudumu wetu.

Kwa habari Zaidi kuhusu haki yako kama mshiriki wa utafiti waweza kuzungum na katibu/Mwenye kiti, Hospitali ya Kitaifa ya Kenyatta-Kamati ya maadili na utafiti Chuo Kikuu cha Nairobi, Nambari ya simu 2726300 Ext. 44102 Barua pepe:uonknh_erc@uonbi.ac.ke.

Wahudumu watakulipa hela zako ukishatumia nambari hizi iwapo mawasiliano yatahusu utafiti

.

CHAGUO LAKO LINGINE NI LIPI?

Uamuzi wako wa kushiriki katika utafiti huu ni wa hiari.Una ruhusa ya kukataa kushiriki katika utafiti na waweza kujiondoa katika utafiti bila hasara yoyote na bila kukiukwa kwa haki yako.

FOMU YA RIDHAA

Kauli ya mshiriki

Nimeisoma fomu hii ya ridhaa ama nimesomewa ujumbe. Nilipata fursa ya kujadiliana kuhusu utafiti huu na mtafiti. Maswali yangu yamejibiwa kwa lugha ambayo naielewa. Nimeelezewa manufaa na hatari ziliwepo. Naelewa kuwa ushiriki wangu kwa utafiti huu ni wa hiari na naweza kujiondoawa wakati wowote.Nimekubali kwa hiari kushiriki katika utafiti huu.

Naelewa juhudi zitafanywa ili kuuhifadhi habari yangu wa kibinafsi.
Kwa kutia sahihi fomu hii ya ridhaa, sijaiacha haki zangu kisheria kama mshiriki katika utafiti.

.

Nimekubali kushiriki katika utafiti huu:	**Ndio**	**La**
Nimekubali mate yahifadhiwe yatumike baadaye:	Ndio	La
Nimekubali kupeana nambari za simu ili nifuatiliwe:	Ndio	La

Jina la mshiriki lililochapishwa:

_____

Sahihi ya mshiriki / alama ya kidole _____Tarehe _____

Shahibi signature _____ Tarehe _____

Kauli ya mtafiti

Mimi, ambaye nimetia sahihi, nimetoa maelezo kamili kuhusiana na utafiti huu kwa mshiriki ambaye ametajwa hapo juuna naamini ya kwamba mshiriki ameelewa na akatoa ridhaa yake kwa hiari.

Jina la mtafiti: Dr.Parina. Patel **Tarehe**: _____

Sahihi

_____

Kazi yake katika utafiti: Mkuu wa uchunguzi

Kwa habari zaidi zungumza na

Mkuu wa Uchunguzi

Dr. Parina Patel

Shule ya kisayansi ya meno, Chuo Kikuu Cha Nairobi,

Nambari ya simu: 0721365744.

Msimamizi mkuu

Lead Supervisor

Dr. Wetende Andrew

Unit Head- Head of Periodontology

Unit of Periodontology/Community and Preventive Dentistry, Department of Dental Sciences, Faculty of Health Sciences, University of Nairobi

Katibu/ Mwenyekiti ,

Hospitali ya Kitaifa ya Kenyatta-Kamati ya maadili na utafiti Chuo Kikuu Cha Nairobi,

Nambari ya simu. (254-020) 2726300-9

Barua pepel: uonknh_erc@uonbi.ac.ke.

Appendix 5: Screening Form

Screening Instructions

1. Only proceed with screening after consent
2. Use a language the participant can understand use translator if necessary

Serial number; …… Age (Years)…… Date …….

Consent to screen; Yes…….. No……...

CRITERIA	**YES**	**NO**
Individual below the age of 18 years.		
Individual who are unable to gargle or spit		
Patients receiving anti-viral medications		
Individuals allergic to any of the active ingredients and have had a history of allergies to PI or CHX mouthwashes.		
Individuals who have thyroid diseases or current radioactive iodine treatment		
Individual who has a concurrent life-threatening systemic illness		
Female patients who are pregnant or lactating		

Appendix 6 : Questionnaire /Biodata Form

EFFICACY OF SELECTED MOUTHWASHES ON SARS-COV-2 VIRAL LOAD USING SALIVARY TESTS IN A HOSPITAL-BASED POPULATION

1. Date.....
2. Saliva sample/ serial number.....
3. Age (Years).....
4. Gender: Male..... Female.....
5. Residence.....
6. Highest Level of Education

   Primary ..... Secondary..... Tertiary.....
7. Vaccination status

a. Vaccinated .....

Vaccine type	**Doses (Date received)**		
	1st Dose	**2nd Dose**	**Booster**
Pfizer			
Oxford/AstraZeneca			
Moderna			
Sinopharm			
Jhonson and Jhonson			

b. Not vaccinated .....

Appendix 7: Laboratory Form

SERIAL NUMBER	DATE OF SAMPLE COLLECTION	SAMPLE (Ct VALUES)	
		T_0	T_1