AN INVESTIGATION OF DIAPHRAGM FUNCTION IN COVID-19 PATIENTS AT A NATIONAL COVID REFERRAL CENTRE (KUTRRH)

A thesis submitted in partial fulfillment of the requirements for the award of the Master of Science Degree in Medical Physiology.

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DECLARATION

I hereby declare that this thesis is my original work and to the best of my knowledge has not been presented elsewhere for approval and for the award of a degree, diploma, or certificate. I further declare that all material cited in this proposal that is not my own work has been duly referenced.

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LIST OF ABBREVIATIONS AND ACRONYMS

ACE2 - Angiotensin converting enzyme 2

COVID-19 - Coronavirus Disease 2019

CMAP - Compound muscle action potential

NCS- Never conduction studies

EXdi - Excursion

KUTRRH - Kenyatta University Teaching Research and Referral Hospital

MIP - Maximal inspiratory pressure

SARS-CoV-2 - severe acute respiratory syndrome corona virus

TFdi - Inspiratory diaphragm thickening fraction (TFdi)

Tdi - Diaphragm thickness

UON - University of Nairobi

TV - Tidal volume

IRV - Inspiratory reserve volume

ERV - Expiratory reserve volume

RV - Residual volume

IC - Inspiratory capacity

ICU-Intensive care unit

FRC - Functional residual capacity

VC - Vital capacity

TLC - Total lung capacity

FVC - Forced vital capacity

FEV1 - Forced expiratory volume in 1st second

TR-Thickening ratio

NACOSTI-National Commission for Science Technology and Innovation

WFN-World Federation of Neurology

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DEDICATION

To my wonderful children- Levi and Nina

To my wonderful Parents- Mungai Ndogo and Jedidah Wanja

ABSTRACT

Background: Long COVID is a debilitating multisystem condition consisting of signs and symptoms that continue or develop after acute COVID-19 infection and are attributed to multiple overlapping mechanisms. The main symptoms of long covid in Africa include Fatigue (41%)confusion (40%) and dyspnea (25%).Dyspnea can result from diaphragm weakness due to the effects of Covid-19 on the diaphragm itself or the phrenic nerve that innervates it. The present study investigated the morphological and functional characteristics of the diaphragm in recovered mild Covid-19 patients.

Objective: To investigate effect of Covid-19 on diaphragm function and morphology and contribution to long covid.

Study setting: Kenyatta University Teaching Research and Referral Hospital (KUTRRH)

Materials and methods: The study was performed on patients that had mild Covid-19 with positive PCR test and matched controls that did not have any Covid-19 like symptoms during the period of November 2021 to January 2022.A sample population of 80 participants was recruited using a convenience sampling method. All the subjects were evaluated by use of Phrenic nerve conduction studies (NCS) ultrasound and spirometry. The experimental data was statistically analyzed using unpaired student t test with significance level set at $P< 0.05$ using R software (version4.0.2)

Results: The FEV1/FVC ratio was greater than 70% of the predicted value and the FEV1 was reduced in the Covid-19 patients. The diaphragm thickness was maintained. However, the amplitude and area under the curve of the compound muscle action potentials (CMAP) after phrenic nerve stimulation were reduced in the Covid-19 patients.

Conclusion: Mild Covid-19 causes reduced amplitude and area of the CMAP suggesting axonal degeneration. This leads to diaphragm weakness, and reduced FEV1/FVC ratio resulting in restrictive pattern of lung disease and consequently contributing to long Covid respiratory dyspnoea.

CHAPTER ONE

INTRODUCTION

Almost half of recovered Covid-19 patients are unable to return to normal activities two months after diagnosis due to persistent symptoms such as dyspnea and fatigue (Azer, 2020). Long covid is a debilitating multisystem condition comprising of signs and symptoms that continue or develop after acute Covid-19 infection and are attributed to multiple overlapping mechanisms. (Davis et al., 2023). While these symptoms could be due to a direct insult to the lung parenchyma, they could also arise from underlying respiratory neuromuscular weakness. SARS-CoV-2 uses the angiotensin converting enzyme 2 (ACE2) as a functional receptor to enter pulmonary alveolar cells and induce acute respiratory distress syndrome (ARDS), a life-threatening condition characterized by poor oxygenation and pulmonary infiltration (Gheblawi et al., 2020). ACE2 receptor is widely distributed and is also found on skeletal muscle and nerve cells suggesting possible effects of SARS-CoV-2 on the diaphragm that warrant further investigation (Ramani et al.,2021)

PROBLEM STATEMENT

Covid-19 Patients continue to experience effects of the illness for weeks after testing negative for SARS-CoV-2 (Shi et al., 2021). About 87.4% of patients who had recovered from the disease, reported persistence of at least one symptom, majority being fatigue and dyspnea (Carfì et al., 2020). patients face challenges in resuming regular activities even with normal cardiac and pulmonary function tests and. This underscores the need to investigate diaphragm weakness secondary to Covid-19 infection as a possible contributing factor to dyspnea symptoms associated with long covid.

JUSTIFICATION

There are More than 651 million long covid patients worldwide (Davis et al.,2023). The increasing number of people with long covid is becoming public health concern. The mechanism behind this persistence has not been well elucidated (van Kessel et al., 2022). The diaphragm weakness resulting from Covid-19 infection may contribute to post-infection respiratory sequelae (Shi et al., 2021). There is limited data about diaphragm function in recovered Covid-19 patients in the Kenyan population.

RESEARCH QUESTION

What are the effects of mild Covid-19 on the thickness and contractility of the diaphragm?

HYPOTHESES

Null hypothesis (H0): Covid-19 has no significant effects on the thickness and contractility of the diaphragm in recovered mild Covid-19 patients.

Alternative hypothesis (HA): Covid-19 has significant effect on the thickness and contractility of the diaphragm in recovered mild Covid-19 patients

OBJECTIVES

Overall Objective

To assess the morphology and function of the diaphragm in recovered mild Covid-19 patients at Kenyatta University Teaching Research and Referral Hospital.

Specific Objectives

- To assess the diaphragm strength through phrenic nerve compound muscle action potential (CMAP).
- To determine the thickness of the diaphragm using Ultrasonography.
- To determine diaphragm excursion on ultrasound
- To assess the FVC and FEV1/FVC ratio using spirometry.

CHAPTER TWO: LITERATURE REVIEW

Coronavirus disease is caused by a novel coronavirus known as Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) (David J, Cennimo, 2023). This novel coronavirus was discovered in December 2019 in the city of Wuhan in the Hubei province of China (Parasher, 2020). it was postulated to be initially a zoonotic outbreak followed by community spread of the disease via human-to-human transmission (David J, Cennimo, 2023; World Health Organization, 2020). The horse-shoe bat is thought to be the natural host of SARS-CoV-2 with several intermediate hosts including pangolin from which it is transmitted to humans. Direct contact with respiratory droplets from an infected person leads to community human to human transmission (Azer, 2020; Ouassou et al., 2020; Parasher, 2020).

Corona viruses are RNA positive-sense, enveloped and non-segmented viruses that can infect mammals including humans (Huang et al., 2020). The SARS-CoV-2 virus associated outbreaks are more lethal with prolonged residual effects Compared to outbreaks associated with SARS-CoV-1 in 2003 and Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012 (Huang et al., 2020). The virus binds to host ACE-2 receptors and enters the pulmonary epithelial cells through endocytosis. It then replicates and forms new viral particles intracellularly that invade the adjacent epithelial cells (Azer, 2020; Bernstein et al., 2018). The average incubation period is 5 days, and almost all people who develop symptoms do so within 12 days ref. The most common symptoms are fever, dry cough, and shortness of breath ref. Although most people with Covid -19 recover within weeks of illness, some experience post-covid conditions which form a wide range of new, returning, or ongoing health problems four or more weeks after first being infected (Farr et al., 2021). In a recent study, nearly half of post- Covid -19 patients were not able to return to work 60 days after hospital discharge and most of them reported persistent shortness of breath, cough, and fatigue post-covid (Farr et al., 2021). Breathing involves the lungs, respiratory muscles and the nervous system and despite the symptoms persisting due to damage to the lung parenchyma it is possible that respiratory neuromuscular involvement to these symptoms (Farr et al., 2021). These post- Covid -19 conditions are known as long covid, long-haul covid, post-acute Covid -19, long-term effects of Covid -19, or chronic Covid -19 (Farr et al., 2020).

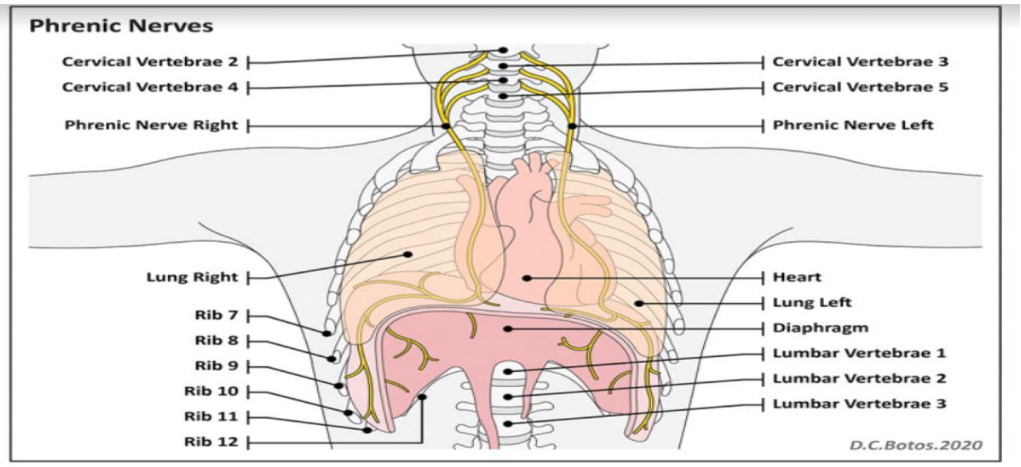
Although several nerves and muscles support breathing, the diaphragm, a thin dome-shaped sheet of muscle, is the main muscle for breathing and the phrenic nerve is its primary motor supply. This nerve also supplies sensory innervation to the diaphragm providing information about feelings or

pain in the central part of the diaphragm, the pleura, and the pericardium or sac that surrounds your heart (Oliver and Ashurst, 2022).

There are two phrenic nerves innervating the right and left side of the diaphragm. They originate from cervical nerves, C3, C4, C5, on each side of the spinal cord. Their path through the neck and chest is similar, with a bit of difference due to the positioning of the heart. They run down the chest close between the lungs and heart to each side of the diaphragm below the lungs (Oliver and Ashurst, 2022). If the phrenic nerve loses some or all function, the diaphragm cannot be pulled down to let enough air into the lungs and the ability to breathe. Injury to these nerves can occur from surgery, blunt or penetrating trauma, metabolic diseases e.g., diabetes, viral infections, direct tumor invasion, neurological diseases e.g., cervical spondylosis and multiple sclerosis, myopathy, and immunological disease e.g. Guillain-Barre syndrome (Mandoorah and Mead, 2022).

Early symptoms of phrenic nerve dysfunction include fatigue on exertion, exercise intolerance, difficulty in breathing when lying down which improves on sitting up, and fatigue (Dubé and Dres, 2016).

Figure 1: Depicts the course of and anatomic relations of the left and right phrenic nerves (Patel et al.,2021)



In normal breathing at rest, inspiration is active, but expiration is passive. During inspiration air is taken into the lung when the phrenic nerve stimulates the diaphragm to contract. This forces the

abdominal contents downward, and the vertical dimension of the chest cavity is increased. The rib cage moves out at the same time (Betts et al., 2013). The action of the diaphragm is assisted by external intercostal muscles, which connect adjacent ribs and slope downward and forward. When these muscles contract, the ribs are pulled upward, thus increasing both the lateral and anteroposterior diameters of the thorax (Betts et al., 2013). However, paralysis of the intercostal muscles alone does not seriously affect breathing, because the diaphragm is so effective. Accessory muscles of inspiration include neck muscles, which assist inspiration during vigorous exercise. During expiration the phrenic nerve relaxes and so does the diaphragm and air is expelled from the lungs. The diaphragm separates the thoracic and abdominal cavities. The costal part of the diaphragm is the major muscle for inspiration and is composed of fatigue-resistant type I fibers and fatigable type II fibers (Dubé and Dres, 2016; Rampon, 2020). The type I fibers in the adult human diaphragm is used during quiet breathing and composes over half of the fibers, while type II fibers are recruited for increased oxygen demand (Dubé and Dres, 2016; Rampon, 2020). Central impulses coordinate contraction and relaxation of the diaphragm that increases and decreases the thoracic volume respectively. On average Humans cycle 5-10 liters of air through the lungs each minute (Dubé and Dres, 2016; Rampon, 2020). Any decline in its function can reduce alveolar ventilation and hence respiratory efficiency (Shi et al., 2021).

The ACE2 receptors are found on the diaphragm. When the SARS-CoV-2 becomes blood-borne it may bind to skeletal muscle cells such as those of the diaphragm producing cytokines known as myokines. These myokines act as signaling molecules which kick-off an immune response (Mittal et al., 2021) whereby neutrophils, CD8 cytotoxic T-cells, CD4 helper T cells are then recruited and sequestered in the lung (Azer, 2020; Bernstein et al., 2018). This uncontrolled immune response results in organ damage due to autoimmune reactions. Skeletal muscles are more prone to undergo atrophy or loss of function in response to pathological conditions (Huang et al., 2020). With more than double the activation of fibrosis pathway in diaphragm of patients covid 19 patients, they have reduced diaphragm thickening ratio and thickness and this plays a critical role in patients' prognosis. However, this effect remains to be fully deduced in patients who have recovered from mild disease without other preexisting comorbidities (Zambon et al., 2017).
Symptoms of diaphragm weakness include unexplained shortness of breath, recurrent pneumonia, anxiety, insomnia, morning headache, excessive daytime somnolence, orthopnea, fatigue, dyspnea

(particularly on exertion), paradoxical inward motion of the abdomen during inspiration on physical examination (Ricoy et al., 2019). Diaphragmatic dysfunction can be associated with important clinical consequences like sleep disturbances and intolerance to exercise.

Diaphragmatic dysfunction can result from multiple diseases and the severity is determined either by the level of anatomical involvement from the cerebral cortex, the spinal cord, the motor neurons, or the muscles themselves. It could also be one-sidedness or bilaterally. Most frequently the dysfunction arises due to diseases affecting the phrenic nerves or from myopathies affecting the diaphragm itself (Patel et al., 2022).

This could result from post-infectious inflammatory neuropathy of the phrenic nerve, or possibly direct neuromuscular involvement of the SARS-CoV-2 virus. There is evidence of skeletal muscle and peripheral nervous system expressing the ACE2 receptor to which the SARS-CoV-2 viral structural spike (S) protein binds (Patel et al., 2022).

It is important to evaluate diaphragm function in patients with acute or chronic respiratory symptoms as it provides guidance on management strategies (Patel et al., 2022). Diaphragm weakness is characterized as reduced motion and thinning of the muscle as detected by ultrasound or as reduced capacity to generate pressures in response to a magnetic stimulus of the phrenic nerves in EMG studies and all these result in restrictive lung pattern (Ricoy et al., 2019). Electromyography (EMG) and the stimulation test are very accurate in the assessment of neural and muscular disorders (Wilcox and Pardy, 1989). EMG evaluates muscle activity by recording diaphragm compound muscle action potential (CMAP) action potentials elicited by phrenic nerve stimulation (Luo et al., 1998).

They can be performed using electrical or magnetic stimulators. Electrical stimulators are less expensive and relatively selective, but they cause the patient discomfort. The Phrenic nerve is stimulated at the level of the neck, and the electromyographic activity of the diaphragm is registered to measure phrenic nerve latencies and amplitudes of muscle compound action potentials (Ricoy et al., 2019). In some neuromuscular disorders such as demyelinating polyneuropathies, latencies are delayed due to slow phrenic nerve conduction. Prolongation of latency beyond the upper limit is strong evidence of a phrenic neuropathy as a cause of diaphragmatic weakness. Normal latency in healthy adult averages 6-8 ms (Ricoy et al., 2019; Wilcox and Pardy, 1989). The amplitude is proportional to the number of nerve fibers activated during stimulation. A reduction in compound diaphragm action potential (CDAP) in comparison

to normal controls has been found in patients with a variety of neuropathies. Normal amplitude values in healthy adult average 500-800 mV (Ricoy et al., 2019; Wilcox and Pardy, 1989) There are specific treatments when the etiology of the weakness or paralysis is known. Some causes due to infectious and metabolic processes are reversible and can improve spontaneously through noninvasive physical therapy to strengthen the diaphragm and to increase engagement of the other muscles involved with breathing, such as the intercostal muscles, scalene muscles of the neck, and abdominal muscles to pull the diaphragm downward (Reeve Foundation, 2021; Ricoy et al., 2019; Wilcox and Pardy, 1989).

Diaphragm ultrasound is a reliable non-invasive tool in detecting diaphragm dysfunction and has a sensitivity of (93%) and specificity (100%) for detecting diaphragmatic neuromuscular disease (Santana et al., 2020). Diaphragm weakness or dysfunction is characterized as reduced motion and thinning of the muscle as detected by ultrasonographic measurements of diaphragmatic thickening fraction (DTF) of less than 20–30% during tidal breathing and a diaphragmatic excursion less than 10 mm from end-expiration to end-inspiration. Diaphragmatic thickness values of less than 0.2 cm at the end of expiration are considered to define diaphragmatic atrophy (Mandoorah and Mead, 2022). When there is muscle atrophy, diaphragm thickness decreases, and it does not contract during inspiration. There is also decreased amplitude of diaphragm movement during deep breathing when there is diaphragmatic weakness (Santana et al., 2020; Sarwal et al., 2013).

The diaphragm is visualized in two ways; one to obtain diaphragmatic thickness (Tdi) in B-mode at the zone of apposition, amid the 8th and 10th intercostal space in the mid-axillary or anterior-axillary line, 0.5–2 cm below the costo phrenic sinus (Farr et al., 2021; Patel et al., 2022; Sarwal et al., 2013):and Two Excursion (EXdi) in M-mode via a subcostal anterior approach between the midclavicular and anterior axillary lines, using liver or spleen as acoustic windows. Diaphragm weakness will result in reduced caudal excursion (Schepens et al., 2020; Zambon et al., 2017). To assess the quality of diaphragmatic function, the thickening ratio (TR) i.e. the thickness at end-inspiration divided by the thickness at end-expiration has been determined. The normal value for the TR has been estimated to be between 1.7 and 2. The diaphragm thickness and inspiratory thickening are greater in sitting and standing positions than in the supine position (Fayssoil et al., 2021).

Pulmonary function tests are relevant to the diagnosis of diaphragmatic dysfunction since it results in a restrictive pattern. The FVC may be decreased more as compared to FEV1, thus giving an

FEV1/FVC ratio of more than 70% (Alaparthi et al., 2016; Ranu et al., 2011; Theerawit et al., 2018). There is a decrease in the total pulmonary, vital and functional residual capacities with a decrease of 15–30% vital capacity from the sitting position to decubitus suggesting some degree of diaphragmatic weakness and requiring further examination e.g., stimulation of the phrenic nerve (Ranu et al., 2011).

Diaphragm dysfunction, although uncommon, is frequently unrecognized because appropriate tests to detect its presence are not performed. Dysfunction can also be weakness which implies decrease in strength or paralysis which is an extreme form of diaphragmatic weakness. Diaphragmatic dysfunctions should be treated in experienced centers, with access to diaphragmatic ultrasonography, phrenic stimulation, pacemaker placement, and surgical experience (Ricoy et al., 2019).

CHAPTER THREE: MATERIALS AND METHODS

STUDY DESIGN

This was a case control study the on patients who got mild covid-19 and controls who did not have any Covid-19 like symptoms.

STUDY SETTING

The study was carried out at Kenyatta University Teaching, Research and Referral Hospital (KUTRRH). As of March 2020, the facility has been gazetted by the Government of Kenya as a key covid-19 treatment and isolation facility.

ETHICAL CONSIDERATIONS

Approvals and authorization to conduct the study were obtained from KNH/UON Ethics and Review Committee (KNH/UON ERC), Department of Physiology (UON), KUTRRH Ethics and Research Committee and National Commission for Science, Technology and Innovation (NACOSTI) prior to the start of the study. Pre-consent counseling of all eligible participants was conducted whereby all methods and procedures were explained to the participant, as well assurance of anonymity and that their data would be kept confidential. Participants were enrolled in the study After informed consent. Patient information was treated with utmost confidentiality and was only used for intended purpose for this study. There were no invasive procedures involved in the study and the participants were not subjected to additional costs.

MITIGATION MEASURES AGAINST COVID-19

The researcher and participants were protected against Covid-19 by adhering to the guidelines set by the WHO/Government of Kenya for mitigating the spread of the disease (COVID-19 and your health,2023). They were required to adhere to infection prevention measures such as maintaining proper hand hygiene by washing with soap and water, sanitization and wearing of full PPE while attending to the patients.

STUDY ENROLLMENT

The target population consisted of patients who had positive Covid-19 PCR tests in the period of November 2021 to January 2022 at KUTRRH

STUDY SAMPLE

The study population included all the patients above 18 years' old who tested positive for Covid-19 by PCR at KUTRRH in the months of November 2021 to January 2022.

INCLUSION CRITERIA

The study recruited patients and controls above 18 years of age who gave informed consent to participate, and who had positive Covid-19 PCR test and matched controls never had any signs or symptoms of Covid-19 at KUTRRH and had no prior known comorbidities including neuromuscular disease.

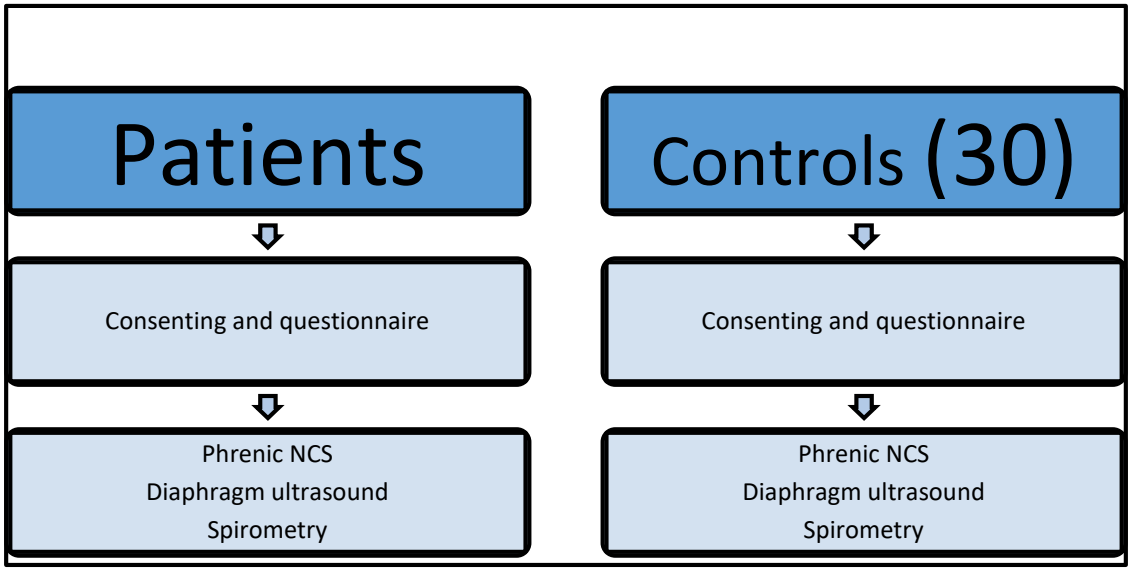
EXCLUSION CRITERIA

Patients with Covid-19 known to have neuromuscular diseases, as previously defined by the World Federation of Neurology (WFN) in 1988, and those with any restrictive lung conditions.

SAMPLING METHOD

The hospital database of positive Covid-19 PCR test results was used to recruit participants. They were contacted by telephone number obtained from the KUTRRH database requesting consent to participate in the study. The study protocol and procedures were explained to the respective participants. participants filled an interviewer guided questionnaire then phrenic NCS, spirometry and Ultrasound measures were taken After verbal informed consent was granted.

Figure 2: Study design and recruitment of participants



NCS, nerve conduction studies

INSTRUMENTS FOR DATA COLLECTION

- Questionnaire
- Phrenic NCS
- Ultrasound
- Spirometer

ADMINISTRATION OF INSTRUMENTS

Administration of the questionnaire, Phrenic NCS, Ultrasound and Spirometry measurements were carried out by the principal investigator.

DATA COLLECTION METHODS

QUESTIONNAIRES

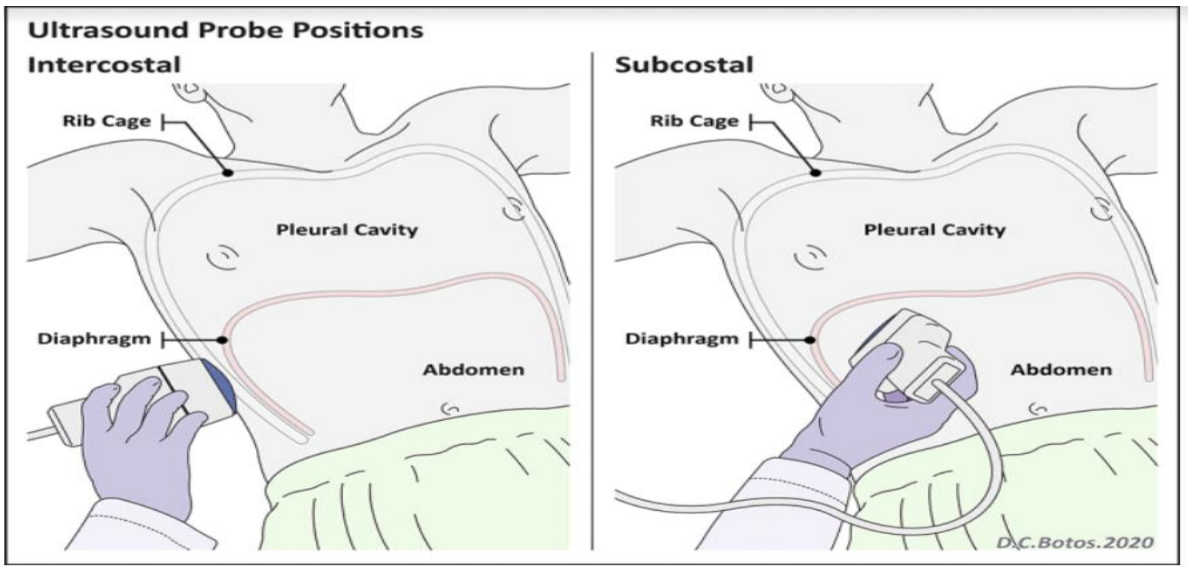
A data abstraction tool was used to collect data from patients: firstly, on personal details of the patient including contact details; vital signs upon data collection; existing neuromuscular disease, restrictive lung diseases (for exclusion); presence or absence of comorbidities; any regular medications/prescriptions; level of physical activity upon admission (minimal, moderate, active); and a checklist of symptoms (initial, resolved and persistent).

After the initial information was gathered, the second section of the document was filled out by the principal investigator on the experiments performed namely: Phrenic NCS, Ultrasound and spirometry measurements of the eligible patients.

DIAPHRAGM THICKNESS USING ULTRASOUND

The principal investigator performed transthoracic diaphragm ultrasound at the bedside with patients in the supine position. The average from three consecutive tidal breaths was used for analysis. Two-dimensional B-mode ultrasound was used to measure diaphragm thickness using intercostal position during inspiration and expiration and M-mode ultrasound tracing was used to obtain the amplitude of diaphragm excursion using subcostal position from the baseline to the point of maximum inspiration using the intercostal approach as previously described (Fayssoil et al., 2021; Patel et al., 2022). Diaphragmatic thickening fraction (DTF) during tidal breathing, excursion from end-expiration to end-inspiration and diaphragmatic thickness at rest were recorded.

Figure 3: Illustration of ultrasound probe positioning for evaluation of the diaphragm utilizing intercostal and subcostal windows. (Patel et al.,2021).



SPIROMETRY

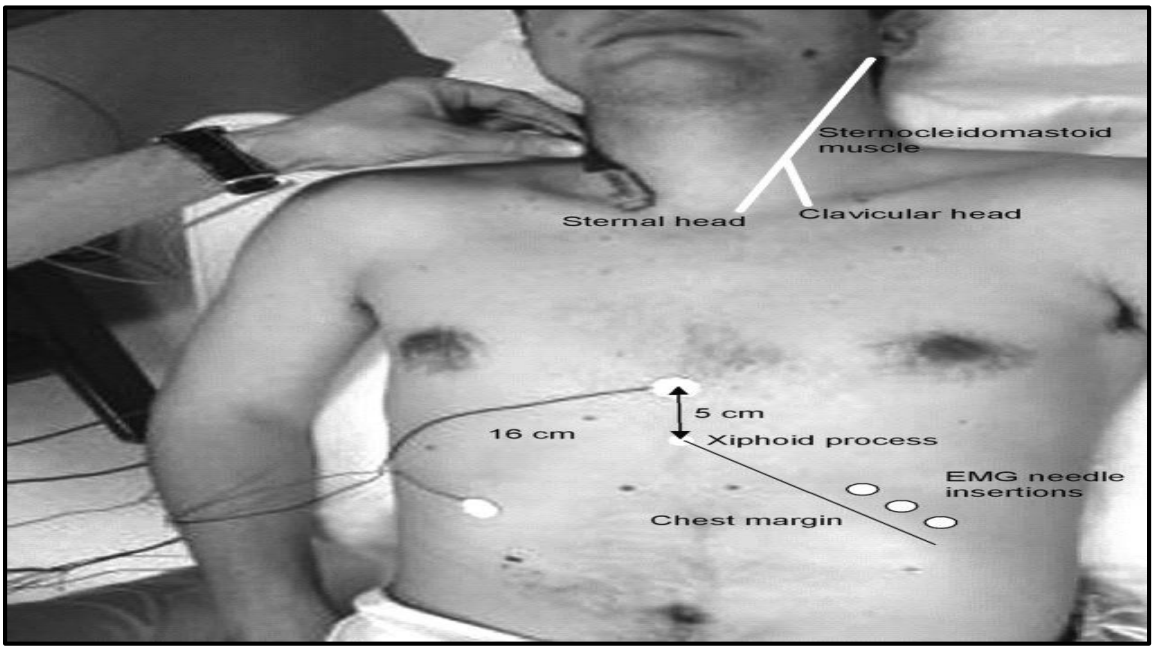
The patients underwent spirometry according to the British Thoracic Society Guidelines (British Thoracic Society, 2013). The forced vital capacity (FVC) and forced expiratory volume in 1st second (FEV1) were measured three times each in sitting and supine positions (Alaparthi et al., 2016).

DIAPHRAGM ELECTROMYOGRAPHY

The diaphragm EMG through stimulation of the phrenic nerve was performed using Nihon Kohden Neuropack M1 MEB-9200 EMG UNIT surface recordings of the diaphragm were obtained using bipolar electrodes placed on the cleaned skin with the subject lying supine. The electrodes were placed in the seventh intercostal space on the right then on the left side of the body at the midclavicular line and ground/reference electrode placed on the sternum. The distance between the two electrodes was less than 2 cm as described by (Bellani *et al.* (2018.) The phrenic nerves were stimulated bilaterally at the posterior border of the sternocleidomastoid muscle at the level of the cricoid cartilage with bipolar surface-stimulating electrodes. The subjects adopted forward flexion of the neck electrical stimulation of the phrenic nerve. The cathode was below the anode. Stimulation at 20mv was performed during at relaxed inspiration and end expiration for all participants for consistency, and also because above that stimulus frequency most subjects were

uncomfortable and declined participation and could not stay still. The latency, amplitude, and area under the curve of CMAP were recorded (Luo et al., 1998).

Figure 4: Diaphragm electromyography lead placement. (Bellani et al., 2018; Luo et al., 1998).



DATA ANALYSIS

Continuous variables i.e., weight, height, and age were analyzed and results presented in a table. Unpaired t-test was done on diaphragm thickness, excursion thickness fraction, CMAP, FVC AND FEV1/FVC ratio of patients and controls with level of significance set at 0.05 and the results were presented in tables and graphs.

CHAPTER FOUR: RESULTS

4.1 DEMOGRAPHIC CHARACTERISTICS.

The sample population was made up of young people between ages of 23-37, majority being females (55%) who did not have any comorbidities. The demographic data is summarized in table 1 below.

Table 1: The baseline characteristics of the subjects who participated in the study.

	All subjects	**Controls**	**patients**	**p-value**
Age (years)	29.93 ± 6.655	29.07 ± 4.479	30.47 ± 7.70	0.9443
Male	45%	65%	35%	--
Female	55%	35%	65%	--
Heart rate (bpm)	76.14 ± 6.521	74.64 ± 6.068	76.96 ± 6.672	0.1224
Respiratory rate (breaths per minute)	17.61 ± 1.372	17.36 ± 1.061	17.75 ± 1.507	0.2035
Systolic blood pressure (mmHg)	120.96 ± 8.934	122.5 ± 6.635	120.1 ± 9.930	0.4116
Diastolic blood pressure (mmHg)	73.84 ± 5.950	73.82 ± 4.234	73.86 ± 6.749	0.7383
Temperature (°C)	36.50 ± 0.241	36.54 ± 0.131	36.48 ± 0.2840	0.8897

SpO_2 (%)	97.59 ± 1.193	97.78 ± 0.832	97.49 ± 1.347	0.4639
BMI (kg/m^2)	41.79 ± 7.357	41.06 ± 7.202	42.23 ± 7.491	0.5124

BMI, Body mass index; SPO2, Oxygen saturation

4.2 DIAPHRAGM EXCURSION, THICKNESS AND THICKENING FRACTION

All the variables met the criteria for normality when assessed using the Shapiro-wilk test. There were no significant differences between the patients and control in all the measured parameters on ultrasound when analyzed using Wilcox test. The ultrasound data is summarized in table 2 and figure 5 below.

Figure 5: The ultrasound variables Patients (cases) and controls

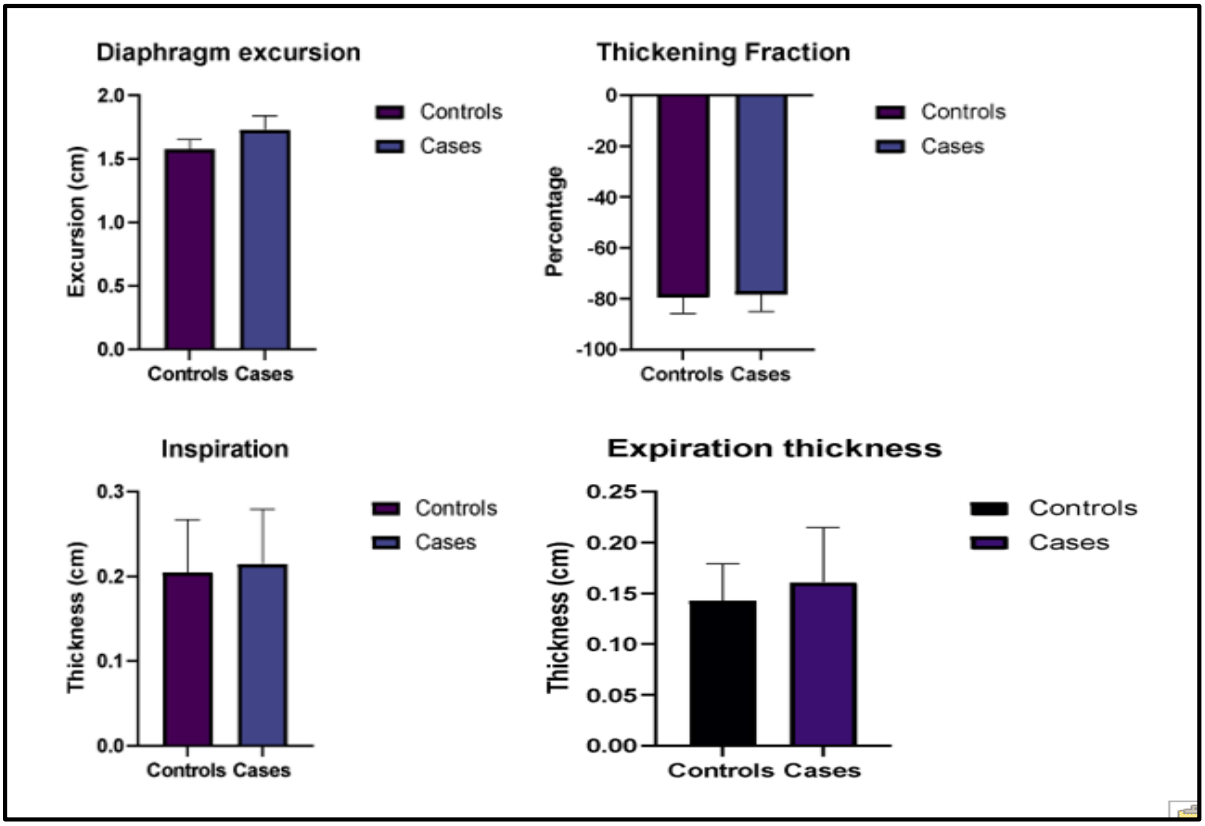


Table 2: Table of the ultrasound variables

	Excursion (cm)	Expiration thickness (cm)	Inspiration thickness(cm)	Thickening fraction (%)
Controls	1.5793 ± 0.4069	0.1431± 0.03626	0.2048 ± 0.06225	-79.51 ± 6.225
Patients	1.7323 ± 0.7857	0.1610 ± 0.0541	0.2152 ± 0.06426	-78.47 ± 6.426
p-value	0.5249	0.1847	0.5308	0.5308

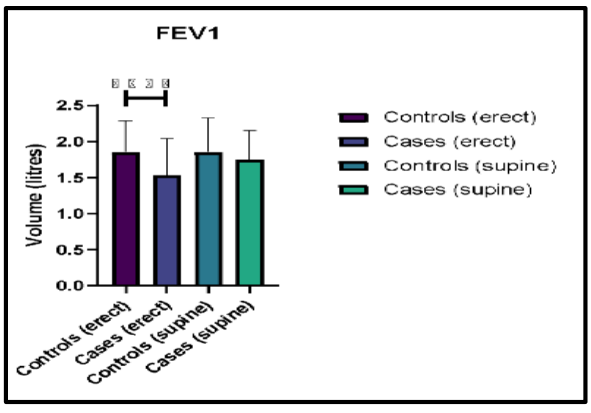
4.3.FVC, FEV1 AND FEV1/FVC RATIO

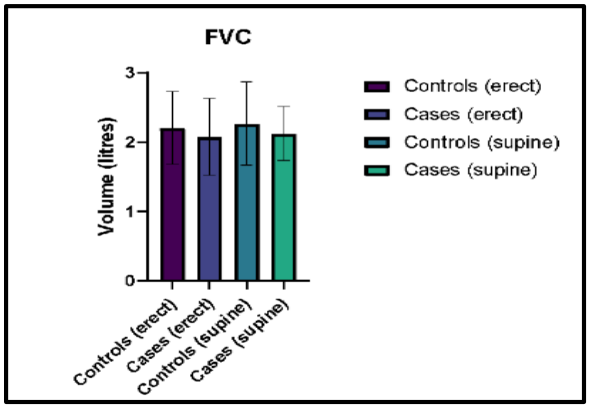
All the variables obeyed the criteria for normality when assessed using the Shapiro-wilk test. There were no significant differences between the FVC of the two groups.

There were significant differences between the two experimental groups in the erect percentage FEV1 of the subjects [86.12 ± 13.61 % (control) vs. 74.85 ± 23.55 % (case): p = 0.0246, Wilcox test]. There were significant differences between the two experimental groups in the erect percentage FEV1 of the subjects [86.12 ± 13.61 % (control) vs. 74.85 ± 23.55 % (case): p = 0.0246, Wilcox test]. The erect percentage FEV1 was significantly reduced in the test group compared to the controls. The spirometry data is summarized in table 3 and figures 6 and 7 below.

Figure 6: The forced vital capacity (FVC) of the Patients (cases) and controls while supine and while standing

Figure 7: The forced expiratory volume (FEV1) of the Patients (cases) and controls while supine and standing





The FEV1/FVC ratio of the patients was (85.79% ± 1.826) which was above 70% of the predicted value that was 60.05%.

Table 3: The FEV1/FVC ratio of the Patients and the predicted value.

	Patients	Predicted
FEV1/FVC ratio (%)	75.54 ± 23.60	85.79 ± 1.826

4.4 PHRENIC NCS

All the variables obeyed the criteria for normality when assessed using the Shapiro-wilk test. There were no significant differences between the two groups in latency and duration of CMAP.

There were significant differences between the two groups in the amplitude and area of CMAP which were significantly reduced in the patients compared to the controls as shown in figures 4-7. The phrenic NCS data is summarized in tables 4,5 and 6 below.

Table 4 The results of the electromyogram of the right phrenic nerve during inspiration.

	Latency (ms)	**Duration (ms)**	**Amplitude (uV)**	**Area (mVms)**
Controls	7.391 ± 2.539	11.857 ± 2.454	456.51 ± 179.1	3.1842 ± 1.4258
patients	8.1325 ± 4.186	10.957 ± 2.2287	362.12 ± 254.95	2.446 ± 1.9186
p-value	0.9402	0.03298	0.03557	0.03049

Table 5 The results of the electromyogram of the right phrenic nerve during expiration

	Latency (ms)	**Duration (ms)**	**Amplitude**	**Area mVms**
Controls	8.322 ± 3.492	11.371 ± 2.164	423.3 ± 161.1	2.787 ± 1.248
patients	7.6256 ± 3.131	10.393 ± 2.642	323.5 ± 240.9	2.220 ± 1.911
p-value	0.1362	0.0905	0.02039	0.03675

Table 6 The results of the electromyogram of the left phrenic nerve during inspiration

	Latency (ms)	**Duration (ms)**	**Amplitude (uV)**	**Area mVms**
Controls	8.092 ± 3.890	11.98 ± 1.534	500.31 ± 174.9	27.75 ± 125.34
Patients	7.725 ± 2.918	10.19 ± 3.043	365.71 ± 266.5	2.343 ± 1.8592
p-value	0.6062	0.004002	0.00658	0.001151

Table 7 The results of the electromyogram of the left phrenic nerve during expiration

	Latency (ms)	**Duration (ms)**	**Amplitude (uV)**	**Area (mVms)**
Controls	7.426 ± 2.681	11.711 ± 1.709	444.10 ± 184.6	3.131 ± 1.560
patients	8.445 ± 4.021	9.965 ± 2.799	326.5 ± 248.3	2.099 ± 1.896
p-value	0.3120	0.005576	0.009534	0.00313

CHAPTER FIVE: DISCUSSION

5.1. Normal thickness, excursion and thickness fraction

There were no significant differences in the excursion, thickness on expiration, thickness upon inspiration and thickening fraction after mild covid-19 infection. This contrasts with previous findings that showed diaphragm dysfunction on ultrasound of covid-19 ventilated and non-ventilated patients (Farr et al., 2021; Law et al., 2022). However, both these studies were conducted in patients with severe disease in which there is pronounced inflammation that can result to fibrosis and atrophy of diaphragmatic muscle (Spiesshoefer et al.,2022, Shi et al., 2021) the inflammation is minimal in mild covid 19, and this may explain why the diaphragm thickness and thus the excursion were normal in these patients.

5.2. Reduced amplitude and area of CMAP

The amplitudes of the compound muscle action potentials (CMAP) were decreased after covid-19 infection. Acute covid-19 peripheral neuropathy affects both small type c and large fiber type a but the small diameter, lightly myelinated type a beta or delta or unmyelinated type c nerves fibers are most susceptible to damage. (Midena et al.,2022). These fibers innervate type I slow and fatigue resistant motor units which are recruited during quiet breathing and thus could result in dyspnea on minimal activity. The amplitude of the action potential is proportional to the number of nerve fibers activated during stimulation and therefore the reduction in amplitude shows that there is possible axonal degeneration that can result in muscle weakness due to a decrease in number of muscle fibers recruited. This may be due to direct invasion of the nerve fibers by SARS-CoV-2 via the ACE2 receptors (Iadecola et al., 2020). It may also occur via invasion through alternative neural receptors such as basigin (BSG; CD147) (Behl et al., 2022) and neuropilin-1 (Cantuti-Castelvetri et al., 2020). These receptors act as docking receptors which are further facilitated by a range of proteases including TMPRSS11A/B, cathepsin B and L, and furin which allow viral cell entry and replication in neurons (Banerjee et al., 2022). This effect on the phrenic nerve may also be due to the inflammatory processes resulting from covid -19. infection. The results of the invasion of SARS-CoV-2 into the phrenic nerve cells may contribute to neuronal death or dysfunction as the cells are hijacked for viral replication (Iadecola et al., 2020).

There is a possibility of the axonal degeneration resulting in a mixed sensory motor neuropathy since the phrenic nerve is a mixed nerve and therefore more studies need to be conducted to determine if the sensory function of the phrenic nerve is also affected (Lefaucheur et al., 2006).

5.3. Reduced FEV1 AND FEV1/FVC ratio above 70%

the FEV1 is usually slightly decreased or stays normal while the ratio of FEV1 to FVC is usually preserved or increased in restrictive pattern of lung disease, (Martinez-Pitre PJ et al.,2023). In this study both the absolute and percentage of the FEV1 for the patients were reduced and the FEV1/FVC ratio were 70% of the predicted value. These findings indicate a restrictive pattern of pulmonary disease that may be secondary to diaphragm muscle weakness as a result of phrenic nerve axonal degeneration (Oliver and Ashurst, 2022).

RECOMMENDATIONS

The present study shows significant bilateral changes to the phrenic nerve that could result to diaphragm weakness. a comprehensive long covid research agenda that builds on the existing knowledge to consolidate the information necessary for decision-making is urgently needed. Additional research is needed to determine whether targeted therapy to promote axonal regeneration e.g., phrenic nerve stimulation and diaphragm muscle exercise to help address exertional dyspnea in patients with long covid. Further studies may elucidate the extent of the damage to the phrenic nerve and the effects of covid-19 infection on the sensory functions of the phrenic nerve.

LIMITATIONS

The sampling method allowed only subjects in the immediate vicinity of the study area to participate in the study, and hence the effects may not be generalized to the entire population. This also yielded a smaller sample size thus the data observed have insufficient power to make conclusive recommendation nevertheless it contributes to the expansive and accelerated research on long covid to be used to improve outcomes for people with long covid.

To mitigate this, patients with positive covid 19 antigens should be included in the study.

CONCLUSION

SARS-CoV-2 shown to significantly reduce phrenic nerve CMAP amplitude and area. This adds to an emerging body of evidence that phrenic nerve and/or diaphragm dysfunction secondary to phrenic nerve axonal degeneration occurs in covid 19 infection. It also highlights the importance of assessing diaphragm function in patients suffering from persistent respiratory difficulties

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APPENDIX

APPENDIX I:

SECTION ONE: STATEMENT OF CONSENT
PARTICIPANT INFORMATION AND CONSENT FORM
ADULT CONSENT FOR ENROLLMENT IN THE STUDY

Title of Study: Investigation of The Effects of COVID-19 Infection on the Diaphragmatic Function Using Electromyography, Sonographic Morphology and Spirometry at Kutrrh a National Covid Referral Centre
Principal Investigator\and institutional affiliation: Dr Mungai Ruth Wanjiru h56/35815/2019.School of Medicine, Department of medical physiology- University of Nairobi

Co-Investigators and institutional affiliation: ……
Supervisors: Dr. F.BUKACHI, Dr. T. KIAMA, Dr. I. ADEMBESA

Introduction:
I would like to tell you about a study being conducted by the above listed researchers. The purpose of this consent form is to give you the information you will need to help you decide whether or not 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 a 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 researchers listed above are doing a study on diaphragm of patients who have been treated for COVID-19 at KUTRRH. The purpose of the interview is to find out whether the participants' diaphragm has been affected in any way by this disease. Participants in this research study was taken through 3 noninvasive procedures on the diaphragm

spirometry, ultrasound and electromyography, asked questions about their health, and further information was deduced from the files.

There were approximately 150 participants in this study conveniently chosen. We are asking for your consent to consider participating in this study.

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 were interviewed by a trained interviewer in a private area where you feel comfortable answering questions. The interview will last approximately 5 minutes. The interview will generally assess your general health details. After the interview has finished, the 3 procedures were carried out and the readings documented.

We will ask for a telephone number where we can contact you if necessary. If you agree to provide your contact information, it was used only by people working for this study and will never be shared with others. The reasons why we may need to contact you include to give you feedback on the overall findings from the study.

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 of our paper records in a locked file cabinet. However, no system of protecting your confidentiality can be absolutely 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.

ARE THERE ANY BENEFITS BEING IN THIS STUDY?

You may benefit by receiving free health information. We will refer you to a hospital for care and support where necessary. Also, the information you provide will help us better understand how to properly manage patients with COVID-19 going forward. This information is a contribution to science and will go a long way in influencing policy on management of covid 19.

WASING IN THIS STUDY COST YOU ANYTHING? No

WILL YOU GET REFUND FOR ANY MONEY SPENT AS PART OF THIS STUDY? Not applicable

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.

This proposal has been reviewed and approved by the KNH/UoN-ERC which is a committee whose work is to make sure research participants are protected from harm. The contact information is given below if you wish to contact any of them for whatever reason;

1. Secretary, KNH/UoN-ERC

P.O. Box 20723 KNH, Nairobi 00202

Tel 7263009

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University of Nairobi research supervisors:

2. Dr. F. BUKACHI

3. Dr. T. KIAMA

4. Dr. I. ADEMBESA

Principal researcher:

1. Dr. Mungai Ruth Wanjiru

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P.O. Box 36153, 00200 Nairobi.

Mobile phone: 0728653564

Kiswahili Version:

UJUMBE WA MSHIRIKI NA FOMU YA IDHINI

IDHINI YA MTU MZIMA YA KUSHIRIKISHWA KWA UTAFITI

Mada: Ustahiki, alama ya uwezekano wa kupona Kisukari baada ya upasuaji na pia uelewaji wa upasuaji kama njia ya kutibu ugonjwa wa Kisukari.

Mtafiti mkuu: Dkt Ruth wanjiru mungai, kutoka shule ya Elimu ya Afya, Idara ya fiziologia, Chuo Kikuu cha Nairobi.

Msaidizi wa utafiti:……..

Waalimu wakuu: Dr. BUKACHI, Dr.T.KIAMA,Dr I. ADEMBESA

Mwanzo

Ningependa kukueleza kuhusu utafiti unaofanywa na watafiti waliotajwa hapa juu. Nia ya fomu hii ya idhini ni kukupa ujumbe ambao utahitaji kukuwezesha kuamua iwapo utakubali kushirikishwa kwa utafiti.

Kuwa huru kuuliza maswali yoyote kuhusu huu utafiti, nini kitakachofanyika kwa utafiti, faida na madhara ya kushiriki kwenye utafiti, haki yako kama mshiriki na chochote kile ambacho hakieleweki. Ukishaelewa kabisa, unawezaamua kushirikishwa kwa utafiti au la. Ukikubali kushirikishwa, nitakuuliza utie sahihi yako kwa hii fomu. Ni vizuri uelewe kwamba i) nia yako ya kushiriki ni kwa hiari yako ii)unaweza kuamua kujitoa kwenye utafiti bila kupeana sababu yoyote iii) kuamua kutoshiriki hakutaadhiri matibabu yako katika hospitali hii. Baada ya kushiriki, tutakupatia nakili ya hii fomu ili uweke kwa rekodi zako.

Tunaweza kuendelea? Ndio La

Utafiti huu umeidhinishwa na The Kenyatta National Hospital-University of Nairobi Ethics and Research Committee protocol No. _____

Utafiti huu unahusu nini?

Watafiti walioandikwa hapa juu kwenye fomu wanafanya utafiti kuhusu matibabu ya ugonjwa wa Covid 19. Nia kuu ni kubaini iwapo COVID-19 ina maafa yeyote kwa kiwambo. Washiriki katika utafiti huu wataulizwa maswali kuhusu uelewaji wao kuhusu Covid 19. Watachukuliwa vipimo vya kilo na kimo, na kisha rekodi za faili zitaangaliliwa.

Kutakuwa na takriban washiriki 219 kwa huu utafiti. Tunakusihi utupe idhini ya kushiriki kwenye utafiti huu.

Ni nini kitakachofanyika ukikubali kushirikishwa kwa huu utafiti

Utaulizwa maswali na mtafiti kwa takribani muda wa dakika 5. Baadaye, utapimwa kilo na kimo na vipimo vingine kusomwa kutoka kwa faili yako. Pia tutauliza utupatie namba zako za mawasiliano iwapo kutakuwa na haja ya kufuatiliza ama kukujulisha kuhusu matokeo ya huu utafiti.

Kuna faida kushiriki kwenye huu utafiti?

Faida ambayo utapata kushiriki kwa huu utafiti ni kwamba utapata maelezo na wosia wa afya njema, na pia utafanikiwa kujua kuhusu njia za kisasa za kutibu ugonjwa wa Covid 19.

Utafiti huu utakugharimu chochote? La

Na iwapo una maswali mengine kwa siku zijazo
Uko huru kuuliza maswali hata bada ya leo. Unaweza kuuliza watafiti kupitia kwa simu/ ujumbe kwa namba zilizipo hapa chini kwa ukurasa.
Kwa ujumbe Zaidi haswa kuhusu haki zako kama mshiriki wa utafiti unaweza kuwasiliana na Mwenyekiti/ Mwandishi Kenyatta National Hospital-University of Nairobi Ethics and Research Committee Telephone No. 2726300 Ext. 44102 email uonknh_erc@uonbi.ac.ke. Iwapo mawasiliano haya yatakuwa kuhusu huu utafiti, watafiti watakurejeshea gharama yako ya kupiga simu.

Maamuzi mengine
Kushirikishwa kwako katika huu utafiti ni kwa hiari yako. Unaweza kukataa kuendelea kwenye huu utafiti na unaweza kujiondoa kwa utafiti huu wakati wowote bila kupoteza huduma yoyote.

Unaweza kuuliza maswali yoyote kuhusu utafiti huu na ukiridhika tafadhali ijaze fomu ya idhini iliyopo hapa chini. Unaweza pia kuuliza swali lolote baadaye kwa kupiga simu kwa mtafiti mkuu ama mkuu wa idara ya upasuaji katika chuo kikuu cha Nairobi ama walimu wasimamizi wa utafiti ukitumia nambari za simu zifuatazo;

Katibu wa utafiti,
Hospitali kuu ya Kenyatta na Chuo Kikuu cha Nairobi,
Sanduku la Posta 20723 KNH,
Nairobi 00202.
Nambari ya simu 726300-9. Walimu wakuu wa Chuo kikuu cha Nairobi:

APPENDIX II: DATA COLLECTION FORM

Serial No ……

Name……

Phone number……

Email……

Occupation……

1. Age (years) [ ]

2. Sex

a. Male [ ]

b. Female [ ]

4. Investigations

a. Full haem gram…….

B. ferritin level…….

c. CRP…….

d. D'Dimers…….

e. UECs …….

5. Measurements

b. Weight (kg) …….

c. Height (m) …….

d. BMI (kg/m2) …….

6. Any chronic illness or neuromuscular disease [yes] [no]

If yes, name specific disease …….

7. Any regular medication you are on [yes] [no]

If yes name specific …….

8. Level of physical activity

A. minimal [ ]

B. moderate [ ]

C. very active [ ]

9. Symptoms

SYMPTOM
INITIAL SYMPTOM
RESOLVED SYMPTOM
PERSISTENT SYMPTOM
FEVER
COUGH
SHORTNESS OF BREATHUSCLE PAIN
LOSS OF TASTE
LOSS OF SMELL
DIARRHOEA
SORE THROAT
SECTION TWO:
To be filled by the radiologist, research assistant and/or primary investigator.

Electromyogram interpretation:
……
……
……
……
……

……
……
……
…….

Filled by:

Name _____

Date _____

Time _____

Spirometry results:

Forced vital capacity (FVC):_____

Forced expiratory volume in 1st second (FEV1): _____

Filled by:

Name _____

Date _____

Time _____

Ultrasound imaging interpretation:

Attach ultrasonogram for record keeping and for reference.

Thickness (cm) _____

Excursion (cm) _____

Filled by:

Name _____

Date _____

Time _____