Potential Effects of SARS-CoV-2 Vaccination on Renal and Thyroid Functions in Apparently Healthy Population in Ibadan, Nigeria

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Certification

This is to certify that Olufisayo Idowu Famuyiwa with matriculation number **LCU/PG/001434** carried out this research work titled "Potential Effects of SARS-CoV-2 Vaccination on Renal and Thyroid Functions in Apparently Healthy Population in Ibadan, Nigeria, in the Department of Biological Science, Faculty of Natural and Applied Sciences, Lead City University, Ibadan, Oyo State, for the award of Doctor of Philosophy Degree (Ph.D.) in Molecular Biology and Genomics and that this has not been previously submitted.

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Dedication

This project work is dedicated to the Almighty God, the God of impossibilities, who made everything easy and cause me to enjoy His favor and love in no measure.

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"Even though the above-mentioned institutions and persons have assisted in the process of this research work, I alone stand responsible for the errors, if any, found in the work

Abstract

Sars-CoV-2 vaccines have proven effective against COVID-19 infection, but their probable adverse effects, especially on renal and thyroid health, remain a cause for concern in some populations. The overall safety and efficacy of currently available Sars-CoV-2 vaccines have been reported, while few studies have documented post-vaccination complications in the kidney and thyroid. There is currently paucity of such complications in our environment. This study assessed the effects of SARS-CoV-2 vaccination on renal and thyroid functions of apparently healthy individuals in Ibadan, Oyo State. It was a cross sectional study carried out following the Oyo State Ethical Board' approval. The study population comprised eighty-two SARS-CoV-2 vaccinated and eighty-six unvaccinated apparently healthy male, and female individuals, ages 18 to 65 years. A structured questionnaire was used to obtain the sociodemographic history of the participants. Ten milliliters of blood samples were obtained for the determination of the serum levels of thyroid stimulating hormone, free tri-iodothyronine and thyroxine, urea, creatinine, C-reactive protein (CRP), glycated hemoglobin, and SARS-CoV-2 antibodies using standard procedures. The data obtained were statistically analyzed, and the results showed that mean levels of the CRP, glycated hemoglobin, kidney, and thyroid function markers in the vaccinated and unvaccinated groups were not significantly ($p > 0.05$) different, and were within the reference ranges. The prevalence of thyroid and renal dysfunction showed that 3.6 % (3) of the vaccinated individuals had slightly elevated CRP (1.3 %), FF4 (1.3 %), and TSH (1.3 %). These values were not statistically significant when compared with the unvaccinated group. The estimated levels of thyroid and renal markers six months post Sars-CoV-2 vaccination in this study were within the reference values, and thus, apparently substantiate the safety of the currently available Sars-CoV-2 vaccines in Oyo State, Nigeria. However, the slightly elevated levels of some of these markers in 3.6 % of the population studied cannot be ignored. It is therefore safe to suggest that a medical follow up beyond six months of vaccination should be encouraged.

Keywords: SARS-CoV-2; COVID-19; thyroid; kidney; vaccination; C-reactive protein

Word count: 300

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List of Acronyms

Abbreviations	Meaning
ACE2	Angiotensin- Converting Enzyme 2
ACEI	Angiotensin Converting Enzyme Inhibitors
Ad-	Adenovirus
AKI	Acute Kidney Injury
AMI	Myocardial Infarction
APC-	Antigen Presenting Cells
ARBs	Angiotensin II Receptor Type1 Blockers
ARDS	Acute Respiratory Syndromes
AT1Rs	Angiotensin II type 1 Receptors
BUN	Blood Urea Nitrogen
C3	Complement 3
CKD	Chronic Kidney Disease
CMs	Convoluted Membranes
CoVs	Coronaviruses
CRP	C- Reactive Proteins

CRS	Cytokine Release Syndrome
CT	C- Terminal
DCT	Distal convoluted tubule
DIC	Disseminated Intravascular Coagulation
DIT	Diiodotyrosine
DM	Diabetes Mellitus
DMSs	Double Membrane Spherules
DMVs	Double Membrane Vesicles
DNA	Deoxyribonucleic Acid
E	Envelope
ER	Endoplasmic Recticulum
ERGIC	Endoplasmic Reticulum to Golgi Intermediate Compartment
FDA	Food and Drug Administration
FT3	Free Triiodothyronine
FT4	Free Thyroxine
GD-	Grave's Disease
GFR	Glomerular Filtration Rate
HBA1C	Glycated Haemoglobin or Glycosylated haemoglobin
HCO_3^-	Bicarbonate
IgA	Immunoglobulin A

IgG	Immunoglobulin G
IL	Interleukins
Kg	Kilogram
LGAs	Local Government Areas
LNP	Lipid Nanoparticles
M	Membrane
MCD	Minimal Change Disease
MERS	Middle East Respiratory Syndrome
MHC-	Major Histocompatibility Complex
MIT	Monoiodotyrosine
mRNA	Messenger Ribonucleic Acid
NaCl	Sodium Chloride
NIH	National Institute of Health
NSPS	Non-structural Proteins
NTD	N Terminal Domain
ORFs	Open Reading Frames
PCI	Percutaneous Coronary Intervention
PCT	Proximal Convoluted tubule
PEP	Phosphor Enol Pyruvate
PKA	Protein Kinase A

PLC	Phosphoinositide Phospholipase
PPE	Personal Protective Equipment
PVN	Periventricular Nuleus
RAAS	Renin- Angiotensin- Aldosterone system
RBD	Receptor Binding Domain
RBD	Receptor Binding Domain
RIG	Retinoic Acid- Inducible Gene
rT3	Reverse Triiodothyronine
RTC	Transcription Complex
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
S- protein	Spike Protein
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SAT-	Sub-Acute Thyroiditis
ssRNA	Single Stranded Ribonucleic acid
STEMI	ST Elevation Myocardial Infarction
TG	Thyroglobulin
TLR	Toll like Receptors
T-lymphocytes	Thymus Lymphocytes
TM	Transmembrane
TMPRSS 2	Transmembrane Serine Protease type 2

TPO-	Thyroid Peroxidase Antibodies
TRH	Thyrotropin Releasing Hormone
TSH	Thyroid Stimulating Hormone
TSH-R	Thyroid Releasing Hormone receptor
UTRs	Untranslated Sections
VEGF	Vascular Endothelial Growth Factor
WHO	World Health Organizatiom
WT	Wild Type

Chapter One

Introduction

1.1 Background to the Study

At the end of December 2019, a few cases of pneumonia of unknown etiology were detected in Wuhan, Hubei Province of China [1]. A few days later, the Chinese authorities identified a novel coronavirus as the etiological agent of the disease [2]. As soon as the complete genome sequence of the 'Wuhan viruses was published online, the structures of various viral proteins were determined. Based on the phylogenetic and taxonomic analysis of the causative agent, the International Committee on Taxonomy of Viruses designated the new virus as 'severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)'[3]. Subsequently, the World Health Organization (WHO) proposed 'COVID-19' as an abbreviation of coronavirus disease 2019. Since the outbreak of the COVID-19 in December 2019, it has continued to spread worldwide and was declared as a "public health emergency of international concern and a global pandemic in March 2020" by the WHO (WHO, 2020).

The overall mortality rate of COVID-19 as at April, 2020 infected people ranges from 0.07–9.3% [3]. SARS-CoV-2 tends to multiorgan involvement due to the widespread expression of angiotensin-converting enzyme-2 receptors, the virus entrypoint [4]. Therefore, COVID-19 causes various clinical scenarios ranging from a flu-like syndrome to more severe conditions such as acute respiratory distress syndrome and death. Non-pharmaceutical measures including physical distancing, proper use of masks, teleworking, isolations and quarantines have been imposed to delay the spread of COVID-19[5]. However, these behavioral measures have unwanted effects, such as a negative psychological impact, major depressions and mental health consequences. Developing a safe and efficient vaccine has been the only promising goal for the successful fight against COVID-19. There were 322 vaccine candidates in development, according to information provided by the WHO. Around 40%

were in clinical development (128 vaccine candidates), while 194 were in preclinical development. The nine leading vaccines – manufactured by Pfizer-BioNTech, Moderna, Gamaleya, Novavax, Oxford-AstraZeneca, Sinopharm, Bharat Biotech, Johnson & Johnson and Sinovac – have been developed based on the use as antigen of the viral S glycoprotein of the wild-type (WT) strain[5].

COVID-19 is mainly spread through the respiratory tract and contact, so the role of mucosal immunity in preventing viral infections was paid more attention. The virus contains four structural proteins. They are Spike S protein, Envelope E protein, Membrane/matrix protein, and Nucleocapsid N protein. The S protein has two subsections, S1 and S2. The S protein binds to specific receptors, causing the virus to infect cells. The neutralizing antibody against the S protein can block this process and prevent the virus from invading. S protein can also effectively stimulate T-cell immune response, so it is the most important target antigen for vaccine design. N and M proteins have also been shown to induce the body to produce an efficient cellular immune response [6]. SARS-CoV-2 is unusual for a respiratory virus that binds to a receptor, angiotensin-converting enzyme 2 (ACE2). ACE2 can be expressed in virtually all organs, but especially in the lungs, gut, and brain.

Therefore, unlike most respiratory viruses, SARS-CoV-2 has a wider biological distribution and may cause considerable damage outside the respiratory system [6]. It adversely affects the genitourinary system, digestive system, circulatory system, and central nervous system. The universality of the distribution of ACE2 receptors leads to multiple changes in symptoms, such as dyspnea, headache, diarrhea, venous thromboembolism, and high blood pressure. The S protein binds to ACE2 on cells to mediate infection. The S1 subunit contains the receptor-binding domain (RBD) and is responsible for initial attachment to the host cells through the ACE2 receptor, while the S2 subunit promotes viral fusion with cells to initiate infection. The

S protein is a frequent vaccine target as it is expected that antibodies binding to the correct epitope on the S protein may be neutralizing and block intercellular viral spread [7].

The vaccines currently under use can be roughly divided into DNA, mRNA, non-replicating viral vector and inactivated vaccines [8]. DNA vaccines can enter cells like viral infections and use the host protein translation system to generate target antigens. As an endogenous immunogen, it can induce humoral and cellular immune responses at the same time. Given the advantages of nucleic acid vaccines, DNA vaccines do not require live viruses, so safety is improved. DNA vaccines insert genes encoding foreign antigens into plasmids containing eukaryotic expression elements and then directly introduce the plasmids into humans or animals, allowing them to express antigen proteins in host cells and induce immune responses to prevent diseases [8]. Compared with DNA vaccines that need to enter the nucleus, mRNA vaccines only need to enter the cytoplasm to achieve target antigens' expression, so they are theoretically safer. Moderna's vaccine, mRNA-1273, specifically encodes the S antigen's prefusion form, including a transmembrane anchor and an entire S1−S2 cleavage site. Non-replicating viral vector vaccines, one of the most explored viral vector options is the Adenovirus (Ad), currently being used by both CanSino and Oxford/ AstraZeneca. Adenovirus (Ad) are the common cold viruses with a double-stranded DNA genome. CanSino is using Ad type 5 (Ad5) and named the vaccine Ad5-nCoV (Hahn and Wiley, 2022). Ad5-nCoV can encode for the full-length S protein of SARS-CoV-2. This gene is derived from the Wuhan-Hu-1 sequence of SARS-CoV-2 and is cloned into the E1- and E3-deleted Ad5 vector together with the tissue plasminogen activator signal peptide. Inactivated vaccines are the most classic form of vaccines [9]. They are easy to prepare and can efficiently cause humoral immune responses. They are often the first choice for new infectious diseases. Inactivated vaccines are mainly obtained through three inactivation methods, such as formaldehyde, β-propiolactone, and ultraviolet. SARS and MERS inactivated vaccines can

cause mice, hamsters, ferrets, and monkeys to produce high-titer neutralizing antibodies. Live attenuated vaccine reduces virus virulence through point mutation or deletion of crucial virus protein but does not affect its immunogenicity and replication ability. This vaccine program has very good immunogenicity and can induce systemic immunity and mucosal immune response, and the immunity is lasting [10].

At present, in the United States, the Food and Drug Administration (FDA) has issued an Emergency use Authorization for the Pfizer-BioNTech (12 years of age and older), the Moderna (18 years of age and older) and Johnson & Johnson (18 years of age and older) vaccines. The Pfizer-BioNTech and Moderna vaccines require two doses, while the Johnson & Johnson vaccine requires only one dose. For the Pfizer-BioNTech vaccine, the two doses are given about 21 days apart. For the Moderna vaccine, the two doses are given about 28 days apart. Second vaccine doses can be delayed if necessary. Current recommendations are to take 2 doses of the same vaccine [9].

Rare adverse events have also been described following the immunisation with COVID-19 vaccines. COVID-19 vaccines are known to cause new-onset or relapsing glomerular diseases due to potent immune dysregulation [11]. Onset of kidney disease symptoms in recently vaccinated patients include foamy urine, hematuria, and edema. IgA nephropathy is the most common glomerulonephritis identified in kidney biopsy, and it is an immune complex disease caused by mesangial IgA1 deposition with or without concurrent IgG and C3 deposits. Although the factors causing the occurrence of IgA nephropathy have not been clearly identified, IgA nephropathy is proposed as a multi-hit disease [11]. If patients who have a genetic predisposition (genetic variation encoding galactosylation) are exposed to subsequent triggering events, such as infection, dietary and environmental stress lead to the production of anti-glycan IgA/IgG, and IgA nephropathy will occur. Klomjit et. al., (2022). reported in their study that COVID-19 vaccination was associated with glomerulonephritis, and 5 out of 13

patients were diagnosed with IgA nephropathy (4 new-onset and 1 relapse). Among them, the partial nephrectomy sample of one patient before vaccination indicated IgA deposition. Therefore, IgA nephropathy after COVID-19 vaccination may be the result of a flare. Moreover, the most common symptom of IgA nephropathy after a COVID-19 vaccination was gross hematuria, and most patients showed a self-limited clinical course without immunosuppression [12].

Minimal change disease (MCD) is a glomerular disease typically characterized by nephrotic-range proteinuria, diffuse foot process effacement in electron microscopy, and no specific abnormalities in light microscopy and immunofluorescence [13]. It has also been confirmed that podocyte injury is caused by various circulating cytokines, such as interleukin (IL)-4, 5, 9, 10, and 13, which are released by activated T lymphocytes. Recently, a Netherlands registry study showed podocyte-associated punctate polyclonal IgG deposits in MCD after COVID-19 vaccination, so B cell activation may also contribute to the onset of MCD to some degree[15]. Typical clinical and pathologic MCD features were confirmed in this case, which may be due to the potent immune response of the COVID-19 vaccine. Biopsy-proven primary podocytopathies were significantly increased after COVID-19 vaccination compared with those before the COVID-19 pandemic, and incidences of MCD have since mainly increased was reported [14]. These findings suggest a potential link between COVID-19 vaccinations and MCD [15].

COVID-19 vaccines have also been implicated in the precipitation of thyroid dysfunction. A cross-reactive immune response against the thyroid cells can result from immune responses to SARS-CoV-2-related proteins [14]. It has been indicated that antibody against SARS-CoV-2 S protein potently reacts with thyroid peroxidase antibodies (TPO). These antibodies may have a role in initiating the autoimmunity via molecular mimicry in susceptible individuals. SARS-CoV-2 S protein, nucleoprotein, and membrane protein, all cross-react with TPO. According

to BLAST matching, a number of TPO-related gene sequences exhibit similarity with gene sequences in multiple SARS-CoV-2 proteins. As a result, antibodies generated against SARS-CoV-2 may contribute to the development of autoimmune thyroiditis [16]. As there is a high similarity between genomic sequences of SARS-CoV and SARS-CoV-2, patients with SARS may also exhibit destruction of thyroid follicular cells. Cross-reactivity of SARS-CoV-2 with thyroid proteins might result in the emergence of autoimmune thyroiditis after COVID-19 vaccination. It should be noted that the existence of molecular similarity does not necessarily lead to autoimmunity. In addition to molecular mimicry, other factors, such as tissue injury, prolonged inflammatory reaction, and genetically predisposed background, may be necessary to cause autoimmune disease. For instance, a Lyme disease vaccine consists an antigenic determinant of Borrelia burgdorferi outer surface protein A, which has high similarity to human lymphocyte function-associated antigen-1, a member of leukocyte adhesion molecules. Despite this similarity raised concern about the safety of this vaccine, there was no indication of the elevated risk of arthritis in people who received the Lyme vaccination [14].

If thyroid autoimmunity is triggered by molecular mimicry between COVID-19 vaccines and thyroid antigens, particularly TPO, it remains to explain why a rise in Hashimoto's thyroiditis has not been reported following vaccination [17]. This is likely due to lack of investigation and under-estimating, because sub acute thyroiditis (SAT) and Grave's disease (GD are characterized by a rapid onset of clinical symptoms that facilitate their diagnosis. However, Hashimoto's thyroiditis has a chronic and slow course, and usually, hypothyroidism (and thus symptoms) occurs years after the appearance of thyroid-related autoantibodies. To establish whether there is an increase also in Hashimoto's thyroiditis occurrence following SARS-CoV-2 vaccination, prospective studies measuring thyroid-related autoantibodies before and

after vaccination as well as comparison with an unvaccinated control group should be conducted [18].

Bystander activation is an antigen non-specific process that results in the activation of autoreactive T cells. In bystander activation, host tissue damage due to immunopathologic responses or infection leads to the releasing of sequestered autoantigens, activating antigen-presenting cells (APCs) and autoreactive Th cells. After that, activated macrophages and autoreactive T cells produce cytokines, which result in the recruitment of more Th cells promoting local inflammation. Abnormal cytokine and chemokine production can lead to aberrant expression of major histocompatibility complex (MHC) class II molecules, contributing to the pathogenesis of viral diseases, probably through the presentation of autoantigens. Imbalances in various T-cell subsets have been related to some thyroid disorders [19].

Furthermore, COVID-19 vaccine-derived S protein can directly bind to ACE2-expressing thyroid cells, leading to thyroid dysfunction[18]. This possible alternative mechanism may explain the occurrence of thyroid dysfunction following vaccination with all types of SARS-CoV-2 vaccines. In addition to the protective target antigen, vaccines may contain other components, such as adjuvants that potentiate the immune response to the antigen, stabilizers, and preservatives, and sometimes traces of antibiotics to avoid bacterial or/and fungal contaminations during the manufacturing process[18]. The vaccine adjuvants (such as aluminum and thimerosal) were linked to autoantibody levels, such as increased anticardiolipin antibodies after influenza vaccination in lupus patients, as well as anti-thyroid and anti-ovarian antibodies after HPV vaccination. The findings presented here indicate that most cases of thyroid abnormalities, including SAT and GD, were observed after vaccination with mRNA-based vaccines. While the novel mRNA-based vaccines of COVID-19 do not contain adjuvants, the mRNA could act as an adjuvant due to its intrinsic immunostimulatory

properties. Indeed, the possible recognition of mRNA by endosomal Toll-like receptors (such as TLR7 and TLR8) and cytoplasmic sensors such as retinoic acid-inducible gene I (RIG-I) potentiate the inflammatory reactions that amplify the autoimmune responses in genetically susceptible people [18].

1.2 Statement of Problem

Various vaccines against COVID-19 have been developed and proven to be effective, but their side effects, especially on kidney and thyroid function, are not yet known in detail. Studies have shown the clinical courses and histopathologic findings of new-onset kidney diseases after COVID-19 vaccination as confirmed via kidney biopsy. The biopsy-proven diagnosis indicated newly developed kidney diseases. IgA nephropathy presenting with painless gross hematuria, minimal change disease presenting with nephrotic syndrome, thrombotic microangiopathy, and cases of acute tubulointerstitial nephritis presenting with acute kidney injury has been reported. Studies has also shown thyroid disorders such as Subacute thyroiditis, Graves' disease, thyroid eye disease, overt hypothyroidism, atypical subacute thyroiditis, and painless thyroiditis with thyrotoxic periodic paralysis after COVID-19 vaccination. Due to limited data available, the specific pathophysiologic link between the incidence of kidney and thyroid dysfunction and COVID-19 vaccination are difficult to confirm.

1.3 Justification of the Study

The response from the worldwide research community to win the COVID-19 pandemic fight has been vigorous, and a multitude of studies regarding the varying aspects of the disease (i.e. prevention, diagnosis, and therapy) have been carried out. Nonetheless, data on the relationship between COVID-19 vaccination and renal and thyroid functions have been emerging,

Up to December 21, 2020, a total of 236 vaccines are being studied, of which 39 have been in clinical trials [20]. However, the vaccines currently in use received emergency use authorization by health agencies but, it is not known whether these vaccines will interact detrimentally with the kidney and the thyroid gland on the long run.

The thyroid gland and the virus infection are known to be engaged in complex interplay via hormones and immunomodulatory signaling molecules. The virus with its associated inflammatory-immune responses could be regarded as a major variable which might affect lifelong renal and thyroid functions, consequently, may suggest the need for monitoring of renal and thyroid function tests after COVID-19 vaccination.

1.4 Aim and Objectives of the Study

Aim

The aim of this study is to determine the effects of SARS-CoV-2 vaccination on the renal and thyroid functions of vaccinated individuals.

The specific objectives of the study are to:

i. estimate the plasma levels of the thyroid function tests -free tri-iodothyronine (FT3), free thyroxine (FT4), thyroid stimulating hormone (TSH), inflammatory marker- c-reactive protein (CRP), renal function parameters- sodium, potassium, chloride, urea, creatinine and cystatin C in the unexposed, unvaccinated and unexposed and vaccinated apparently healthy population
ii. determine if there is thyroid dysfunction in apparently healthy individuals who are vaccinated.
iii. determine if there is renal dysfunction in apparently healthy individuals who are vaccinated.

iv. determine if there is raised inflammatory markers in apparently healthy individuals who are vaccinated.

v. compare the plasma levels of thyroid function hormones, renal function parameters and inflammatory markers in unvaccinated group and those who had been vaccinated.

1.5 Research Questions

i. What are the levels of the thyroid hormones, renal function parameters and inflammatory markers in the unexposed and not vaccinated apparently healthy individuals and unexposed but vaccinated individuals?

ii. Is thyroid gland dysfunction a frequent feature in individuals who had been vaccinated?

iii. Is renal dysfunction a frequent feature in individuals who had been vaccinated?

iv. Are raised inflammatory markers a frequent feature in individuals who had been vaccinated?

v. Are there differences in the levels of the thyroid function tests, renal function tests and inflammatory markers in unexposed, unvaccinated group and the unexposed but vaccinated group?

1.6 Hypothesis

Null Hypothesis (Ho): SARS-CoV-2 vaccination does not affect renal and thyroid function in individuals who had been vaccinated

Alternate Hypothesis (Ha): SARS-CoV-2 vaccination affects renal and thyroid function in individuals who had been vaccinated

1.7 Significance of the Study

This study will make some important contributions to providing data on the possible effect of SARS-CoV-2 vaccination on the renal and thyroid functions of apparently healthy individuals in Ibadan, within 6-8 months post SARS-CoV-2 vaccination. Information from this study will help to know if the COVID-19 vaccinations have any adverse effect on some organs needed for proper functioning of the body. Thus may suggest the need to check the health status of an individual before and after vaccination.

In addition, there is paucity of data on the effect of COVID-19 vaccines on the renal and thyroid functions in our environment. The outcome will assist in giving possible reassurance to the populace that the vaccines are safe and do not have negative consequences on their health, bearing in mind that many people had serious hesitation for the COVID-19 vaccines due to wide belief that the available vaccines received emergency use authorization, hence may pose a lot of genetic dangers. Therefore, the outcome will assist clinicians on whether to check or not the renal and thyroid functions of individual before and after vaccination.

1.8 Scope of the Study

There had been several types of side effects of COVID-19 vaccination since the commencement of the vaccination program ranging from tiredness, headaches, chills, redness or swollen at the site of the injection, but there had been very scarce data on the effect of COVID-19 vaccines on the renal and thyroid function in this environment. Hence, this study aims to determine the effects of SARS-CoV-2 vaccination on the renal and thyroid functions of vaccinated individuals.

The scope of this study is restricted to recruiting a total of two hundred and eighty-four apparently healthy participants (males and females) between the ages of 18- 60 years who had never tested positive to SARS-CoV-2 infection from some Local Government areas in

Oyo state. One hundred and thirty-nine were individuals who had been vaccinated at most eight months prior to sample analyses and tested negative to SARS-CoV-2 infection using Real–Time- polymerase chain reaction (RT-PCR) Government testing sites in Ibadan. These individuals were compared with a matched cohort of one hundred and forty-five apparently healthy individuals, negative and unvaccinated to SARS-CoV-2 infection after informed consent was given. Individuals with previous history of thyroid dysfunction, diabetes, hypertension and renal dysfunction were excluded from the study. The study was conducted for a period of six months.

1.9 Limitation of the Study

A major limitation faced during this study was that many of the participants were very sceptical and it took much drive before giving consent to be part of the study. It was a lot of effort going almost every day to the COVID-19 testing centres to recruit those who tested negative.

Another challenge is the fear some still have for blood collection, thus making it a very tiring exercise. Some individuals who had agreed initially to be part, dropped out at the point of blood collection. Another important aspect of the limitation is peoples' hesitancy to COVID-19 vaccination, this made the data and sample collection from the vaccinated group very tedious and more time consuming.

1.10 Operational Definition of Terms

SARS-CoV-2: The virus that causes a respiratory disease called coronavirus disease 19 (COVID-19). SARS-CoV-2 is a member of a large family of viruses called coronaviruses. These viruses can infect people and some animals. SARS-CoV-2 was first known to infect people in 2019. The virus is thought to spread from person to person through droplets released when an infected person coughs, sneezes, or talks. It may also be spread by touching

a surface with the virus on it and then touching one's mouth, nose, or eyes, it is also called severe acute respiratory syndrome coronavirus 2.

COVID-19: Coronavirus disease 2019 (COVID-19) is a contagious disease caused by the virus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The first known case was identified in Wuhan, China, in December 2019. The disease quickly spread worldwide, resulting in the COVID-19 pandemic.

ACE-2 Protein: Angiotensin converting enzyme 2 (ACE-2), also known as ACEH (ACE homolog), is an integral membrane protein and a zinc metalloprotease of the ACE family. ACE-2 also serves as the receptor for the SARS-CoV and SARS-CoV-2 viruses. Binding of the S1 domain of the SARS Coronavirus Spike protein to ACE-2 initiate viral entry into the host cell. Recently, this interaction between SARS-CoV-2 and ACE-2 has become an area of intense therapeutic interest to develop treatments against COVID-19.

Minimal Change Disease: Is a kidney disease in which large amounts of protein is lost in the urine. It is one of the most common causes of the Nephrotic Syndrome.

Thyroid Peroxidase (TPO): Thyroid peroxidase is an enzyme normally found in the thyroid gland. TPO plays an important role in the production of thyroid hormones. Thyroid peroxidase is a frequent epitope of autoantibodies in autoimmune thyroid disease, with such antibodies being called anti-thyroid peroxidase antibodies (anti-TPO antibodies). This is most commonly associated with Hashimoto's thyroiditis.

Sub-Acute Thyroiditis (SAT): This is also known as De Quervain thyroiditis, granulomatous thyroiditis, or giant cell thyroiditis) is inflammation of the thyroid characterized by a triphasic course of transient thyrotoxicosis, followed by hypothyroidism, followed by return to euthyroidism.

Graves 'Disease: Graves' disease is an immune system disorder that results in the overproduction of thyroid hormones (hyperthyroidism). Thyroid hormones affect many body systems, so signs and symptoms of Graves' disease can be wide ranging. Although Graves' disease may affect anyone, it's more common among women and in people younger than age 40[14].

Renal Function Tests: Renal function tests (RFT) are a group of tests that may be performed together to evaluate kidney (renal) function. The tests measure levels of various substances, including several minerals, electrolytes, proteins, and glucose (sugar), in the blood to determine the current health of the kidneys.

Glomerulonephritis: encompasses a subset of renal diseases characterized by immune-mediated damage to the basement membrane, mesangium, or capillary endothelium, leading to hematuria, proteinuria, and azotemia.

Chemiluminiscence: Chemiluminescence (CL) is defined as the production of electromagnetic radiation (ultraviolet, visible or infrared) observed when a chemical reaction yields an electronically excited intermediate or product, which either luminesces (direct CL) or donates its energy to another molecule responsible for the emission (indirect or sensitized CL).

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Chapter Two

Literature Review

2.1 Conceptual Review

SARS-CoV-2 is the coronavirus responsible for the current COVID-19 pandemic. Coronaviruses (CoVs) are a diverse group of viruses that can cause a variety of respiratory illnesses in people, ranging from the common cold to more serious and uncommon conditions like the severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS), both of which have high mortality rates and were first identified in 2003 and 2012, respectively [1]. The potential of a global pandemic from coronaviruses has long been recognized. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the sixth coronavirus to be found in humans in the past 20 years [2,3]. The great majority of human viruses, as well as all prior human coronaviruses, have zoonotic origins. There are various indications of these earlier zoonotic occurrences in the emergence of SARS-CoV-2. The SARS-CoV that infected people in Foshan, Guangdong province, China, in November 2002, and again in Guangzhou, Guangdong province, in 2003, clearly resembles it [4]. A SARS-CoV intermediary host is anticipated between the bat and the human host, even though the bat (*Rhinolophus affinis from Wunnan*) may be regarded as a natural reservoir for this category of coronaviruses. Pangolins may act as an intermediate host for the SARS-CoV2-like virus due to genomic similarities [5]. SARS-CoV2 shares 79.8% genetic identity with the original SARS-CoV virus and 59.1% with the MERS-CoV virus [5, 6].

The four genera of SARS-CoV-2 are alpha, beta, gamma, and delta- CoVs. All SARS-CoV-2 that are now recognized as being able to harm humans are either alpha- or beta. Many of these SARS-CoV-2 can also infect a variety of animal species. In 2002, SARS-CoV-2 infected civet cats and humans, while in 2012, MERS-CoV was discovered in dromedary camels and humans [1]. Both SARS-CoV-2 emergence incidents involved live animal markets

and involved species, particularly civets and raccoon dogs [7], which were also offered for sale alive in Wuhan markets in 2019 [8], and which are known to be susceptible to SARS-CoV-2 infection [9]. High levels of immunoglobulin G (IgG) to SARS-CoV-2 were found in animal merchants working, who had not yet been diagnosed with SARS-CoV-2 (13% overall and >50% for traders who specialized in civets) [10].

SARS-CoV-2 from a detailed study has been found to be 96% identical at the whole genome level to the bat SARS like coronavirus strain Bat Covs, RaTG13, making it likely that bats served as reservoir hosts. With many theories not supportive of direct spillover from bats to humans, further investigation was conducted. Pangolins were then reported as potential intermediate hosts after samples were analyzed from Malytan pangolins, an endangered species illegally trafficked into southern China for use in old-fashioned Chinese medicine and as a food source. These were obtained from Guangdong and Guangxi, China during an anti-smuggling operation. Samples from the pangolins showed new coronavirus genomes with 85.5– 92.4% resemblance to SARS-CoV-2. More remarkable was the 97.4% amino acid similarity in the receptor binding domain (RBD) of coronavirus genomes from pangolins compared to SARS-CoV-2. In comparison, the Bat CoV RaTG only had 89.2% amino acid similarity in the RBD with SARS-CoV-2. Up until now, bats and pangolins are the only two mammals known to be infected by SARS-CoV-2-related coronaviruses [5].

2.2 Viral Morphology

On the Vero cells, SARS-CoV-2 was inoculated after being isolated from nasopharyngeal and oropharyngeal samples. Transmission electron microscopy was used to identify SARS-CoV-2 after injected cells were pretreated with 2% paraformaldehyde and 2.5% glutaraldehyde. 3 days after infection, the structure of SARS-CoV-2 was determined by looking at infected cells. With virus particle sizes ranging from 70 to 90 nm, SARS-CoV-2 was found to have a coronavirus-specific shape by electron microscopy under a range of

intracellular organelles, but most notably in vesicles [11]. High sequence similarity has led to speculation that SARS-CoV-2 shares the same structure as SARS-CoV [12]. In host membrane-derived lipid bilayer that encases the helical nucleocapsid containing viral RNA, the coronavirus's surface viral protein spike, membrane, and envelope are embedded [5]. A newer class of medicines for the treatment of COVID-19 may be developed now that the structures of spike and protease of SARS-CoV-2 have been determined[13].

SARS-CoV-2 belongs to the beta-coronavirus group, which also includes MERS-CoV and SARS-CoV. The latter shares ~75–80% of its viral genome with SARS-CoV-2. Beta-coronaviruses have three important envelope proteins: Spike (S) protein, Membrane (M) protein, and Envelope (E) protein. S protein mediates viral attachment to the cell membrane receptor, membrane fusion, and ultimately viral entry into the host cell. M protein, the most abundant membrane protein, together with E protein are responsible for the coronavirus membrane structure. Another component of the beta-coronavirus is the N protein, which is the protein component of the helical nucleocapsid that includes the genome RNA.

The coronavirus genome is between 26 and 32 kb in size and contains 6 to 11 open reading frames (ORFs) that encode 9680 amino acid polyproteins. The first ORF, which encodes 16 nonstructural proteins (NSPS), makes up roughly 67% of the genome. The remaining ORFs code for accessory and structural proteins. The hemagglutinin-esterase gene is not present in the SARS-CoV-2 genome. It does, however, have two flanking untranslated sections (UTRs) at the 358 and 5′ ends of the 265 nucleotides. There were no discernible differences in ORFs and NSPS between SARS-CoV-2 and SARS-CoV, according to sequence variation [14].

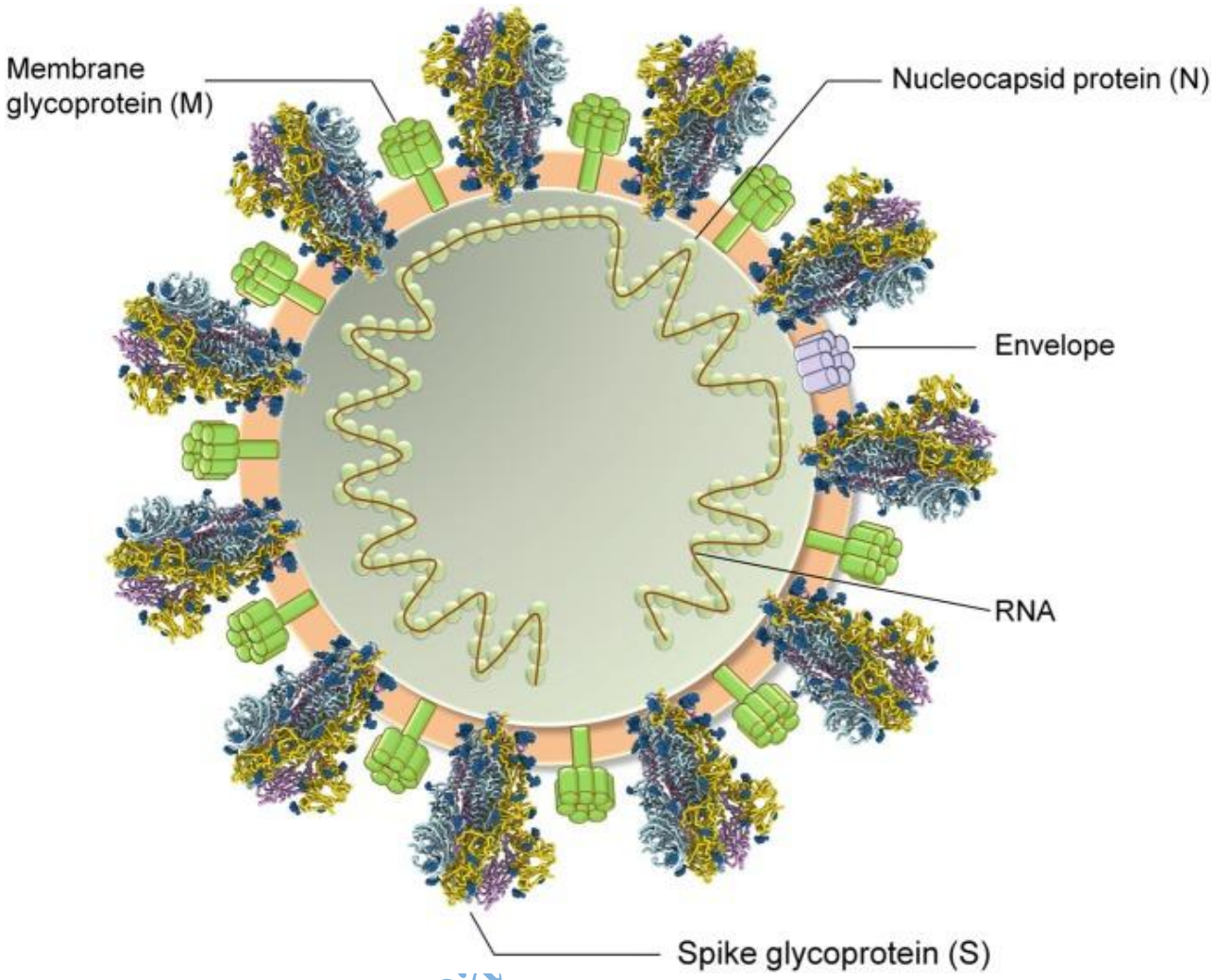

Fig 2.1: SARS-CoV-2's structure. Spike glycoprotein (S), a surface viral protein of SARS-CoV-2, interacts with the cell surface receptor ACE2 through surface viral proteins. The helical nucleocapsid containing the viral RNA is enclosed in a lipid bilayer produced from the host membrane by the viral membrane glycoprotein (M) and envelope (E) of SARS-CoV-2.

Source: Science, 2003[8]

The nsps contains two viral cysteine proteases that are thought to be involved in the transcription and replication of SARS-CoV-2, including RNA-dependent RNA polymerase (nsp12), helicase (nsp13), papain-like protease (nsp3), chymotrypsin-like, 3C-like, or major protease (nsp5), and others [15]. Spike surface glycoprotein (S), membrane, nucleocapsid protein (N), envelope (E), and auxiliary proteins encoded by ORFs are the other four primary structural proteins in addition to nsps. At the N-terminal end of the M protein, which has three transmembrane domains (TM) and a lengthy C-terminal CT domain, is an N-terminal glycosylated ectodomain. For virus morphological development, assembly, and budding, the M and E proteins are necessary, but the S glycoprotein is a fusion viral protein made up of the S1 and S2 subunits. The S1 subunit consists of a signal peptide, an N-terminal domain (NTD), and a receptor-binding domain (RBD), and it shares 70% of its sequence with human SARS-CoV and bat SARS-like CoVs [16].

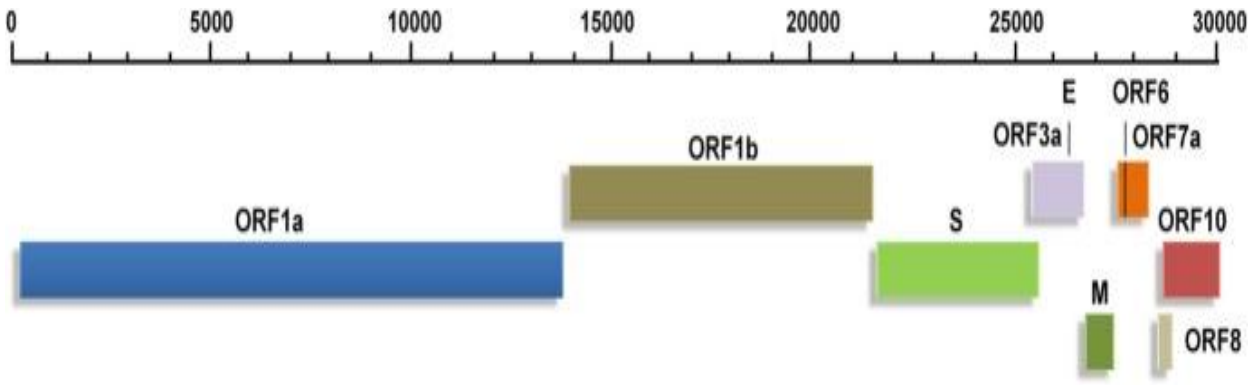

Fig 2.2: SARS-CoV-2 genomic organisation. The coronavirus genome is between 26 and 32 kb in size and contains 6 to 11 open reading frames (ORFs) that code for a polyprotein with 9680 amino acids. While the remaining ORFs code for accessory and structural proteins, the first ORF makes up roughly 67% of the genome and encodes 16 nonstructural proteins (nsps). The nsps contains two viral cysteine proteases, including RNA-dependent RNA polymerase (nsp12), helicase (nsp13), papain-like protease (nsp3), chymotrypsin-like, 3C-like, or major protease (nsp5), and others that are probably involved in the transcription and replication of SARS-CoV-2. The genome also encodes for auxiliary proteins like ORFs and the four primary structural proteins spike surface glycoprotein (S), membrane, nucleocapsid protein (N), and envelope (E).

Source: Current Bology, 2020[5]

2.3 Mode of Transmission

SARS-CoV-2, like other coronaviruses, spreads primarily through respiratory droplets that are infected, with viral infection occurring through direct or indirect contact with nasal, conjunctival, or oral mucosa when respiratory particles are inhaled or deposited on these mucous membranes[17]. The human respiratory tract epithelium, comprising the oropharynx and upper airway, contains most target host receptors. The gastrointestinal system and conjunctiva are both prone to infection and could act as transmission channels [17].

According to earlier research on SARS-CoV-1, respiratory droplets are the main means by which beta-coronaviruses are spread[18]. Droplet transmission occurs through direct contact when a person is exposed to infective respiratory droplets when they are within 1 m of someone with respiratory symptoms including coughing and sneezing. Being within this distance puts the individual at risk of having their mucous membranes, including their mouth, nose and eyes, exposed to the droplets. Transmission can also occur through indirect contact by way of fomites on surfaces in the immediate environment around the infected person. Airborne transmission may be possible when aerosol-generating procedures are performed including endotracheal intubation, cardiopulmonary resuscitation, administration of nebulized treatments, and others. Transmission of the virus can occur in the pre-symptomatic incubation period. A study in a nursing home showed that more than half of the residents with positive test results for SARS-CoV2 infection were pre-symptomatic and most likely contributed to transmission. Asymptomatic transmission (i.e., in patients who never develop symptoms) can also occur as suggested in some studies[19].

Another mode of transmission that should be considered is airborne because it can spread viruses deeper into the bronchial and alveolar tissues when inhaled [20]. In bronchoalveolar lavage fluid, sputum, and nasal swabs, viral RNA was detected more frequently in a study of COVID-19 patients from three hospitals in China [21]. In addition, despite only having modest

upper respiratory tract involvement, one of the three people in a research analyzing environmental contamination in symptomatic patients had 87% of his tested room sites, including air exits, return positive for viral RNA. Viral transmission through contagious droplets may be sustained by environmental contamination through airflow [22].

2.3.1 Indirect Transmission

Along with the aerosolization of microbe particles, infection due to contact with contaminated objects or exposure to flies is another possibility. The results of an investigation that assessed the virus's survivability on several inoculation samples' surfaces suggested that indirect transmission via fomite and aerosolization was likely [23]. An early study that tracked COVID-19 patients to determine their environmental exposures inside a mall. According to the data, respiratory droplet transmission alone was not likely to be the cause of viral dissemination. Some subjects' transmission was caused by direct contact with other subjects, by asymptomatic people, or by exposure to contaminated environments [24]. Another study also emphasized the virus' ability to spread on cruise ships, where there have been more than 800 confirmed cases; viral RNA was found in symptomatic and asymptomatic people's cabins despite quarantining efforts, raising doubt about the virus' ability to spread through fomites [25].

In addition, a study also described the environmental viral shedding that was seen in 13 quarantined people in the USA; of the 163 surface and aerosol samples taken, 126 (77.3%) tested positive for SARS-CoV-2 by reverse transcriptase-polymerase chain reaction (RT-PCR). The presence of viral RNA was also detected in 80.4% of all room surface samples, supporting extensive indirect contamination and possibly airborne transmission [26]. Other mode of transmission include:

- Faecal-oral route of transmission.
- Sexual route of transmission.

- Ocular route of transmission.

2.4 Pathogenesis of SARS-CoV2

The host target cell receptor, ACE 2, is where SARS-CoV-2 attaches [27]. Non-specific symptoms like fever, myalgia, headaches, and respiratory symptoms are brought on by the virus's active reproduction and release in the lung cells. The virus temporarily damages the cells in the olfactory epithelium in an experimental hamster model, resulting in olfactory dysfunction, which could account for the brief loss of taste and smell that is frequently observed in covid-19 [28]. The locations of infections and patient symptoms may be explained by the distribution of angiotensin converting enzyme 2 (ACE 2) receptors in various tissues. The ACE 2 receptor, for instance, is located on the epithelium of various organs, including the gut, as well as endothelial cells in the kidney and blood vessels, which may help to explain gastrointestinal symptoms and cardiovascular issues [29]. Patients with SARS-CoV-2 infection have pathological results that are quite like those of patients with SARS-CoV and MERS-CoV.

In addition to liver cell necrosis and myocardial infarction, lymphocytic endotheliitis and Glomerulonephritis has been identified in postmortem pathology examinations of the lung, heart, kidney, and liver in patients who died from covid-19 [27]. These results suggest that, like SARS-CoV-1 and influenzae, the virus directly affects several organs [30].

The structural proteins that this virus encodes include membrane proteins, nucleocapsid proteins, envelope proteins, and spike glycoproteins. It also encodes non-structural proteins, the majority of which make up the viral replication and transcription complex. The structural proteins produce an enveloped virion (or virus particle) that carries viral genomic RNA into the cell together with a lipid bilayer obtained from the host [31]. The spike glycoprotein, which forms trimers on the surface of virions, is the primary determinant of coronavirus tropism [30].

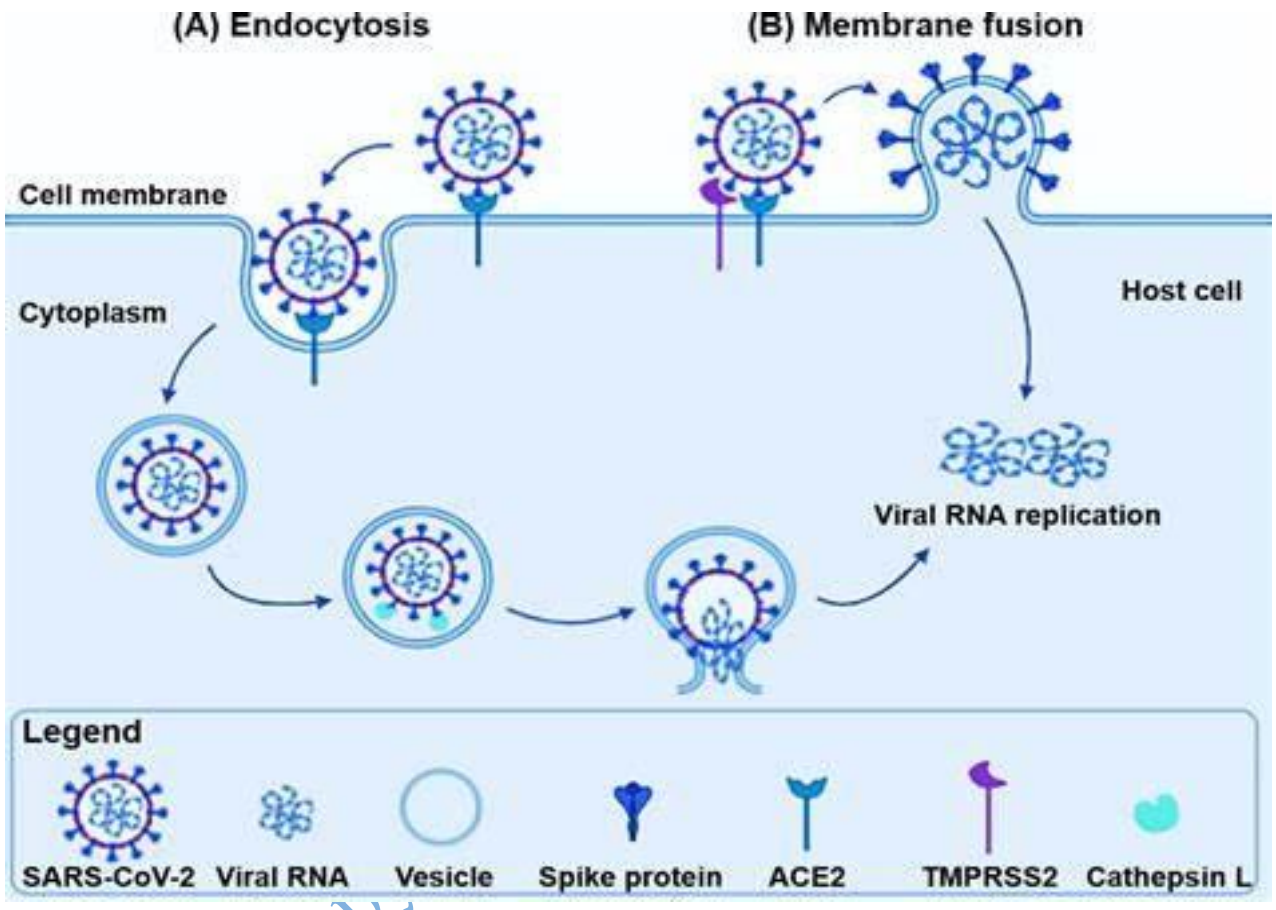

Fig 2.3: Molecular and cellular pathogenesis of SARS-CoV-2

Source: Science, 2003[8]

2.5 Viral Replication

Coronaviruses (CoVs) are a large group of enveloped positive-sense single-stranded RNA viruses. SARS-CoV-2 incubation time has been estimated to be between 5 and 7 days. When released into the cytoplasm of a host cell, most +RNA viruses contain a single-strand positive-sense RNA genome that can be directly translated into viral proteins. Upon synthesis, viral proteins perform a variety of functions, including viral RNA (vRNA) replication, host cell process modification to facilitate virus amplification, and repression of antiviral signaling in the host cell. Following the translation of the incoming vRNA (i.e., the vRNA that infected the host cell), the newly synthesized vRNA-dependent RNA polymerase (RdRp) produces negative-sense RNA (RNA), which is then used as a template for the synthesis of additional +RNAs. These new +RNAs can be encapsulated to form new infectious virus particles or can enter a new round of translation and replication [32]. Because vRNA molecules can participate in multiple processes (translation, replication, and/or packaging), tightly controlled switching between these dynamic processes is likely critical for virus replication. A translation-to-replication switch is required even for incoming vRNA to initiate virus replication in newly infected cells. Although some factors that may contribute to the transition from translation to replication have been identified[32].

The beginning steps of coronavirus infection entail the specific binding of the coronavirus spike (S) protein to the host's cellular entry receptors, which have been identified for several coronaviruses and include angiotensin-converting enzyme 2 (ACE2; HCoV-NL63, SARS-CoV and SARS-CoV-2), human aminopeptidase N (APN; HCoV-229E) and dipeptidyl peptidase 4 (DPP4; MERS-CoV). Entry receptor expression and tissue distribution, in turn, influence viral tropism and pathogenicity. Coronaviruses express and replicate their genomic RNA to produce full-length copies that are incorporated into newly produced viral particles during the intracellular life cycle. Coronaviruses have enormous RNA genomes that are

flanked by 5′ and 3′ untranslated regions that contain cis-acting secondary RNA structures required for RNA synthesis. The genomic RNA has two large open reading frames (ORFs; ORF1a and ORF1b) at the 5′ end that occupy two-thirds of the capped and polyadenylated genome.

ORF1a and ORF1b encode 15-16 non-structural proteins (nsp), 15 of which comprise the viral replication and transcription complex (RTC), which includes RNA-processing and RNA-modifying enzymes, as well as an RNA proofreading function required for the integrity of the >30kb coronavirus genome. ORFs that encode structural proteins are transcribed from the 3′ one-third of the genome, and interspersed ORFs that encode accessory proteins form a nested set of subgenomic mRNAs (sg mRNAs) [31].

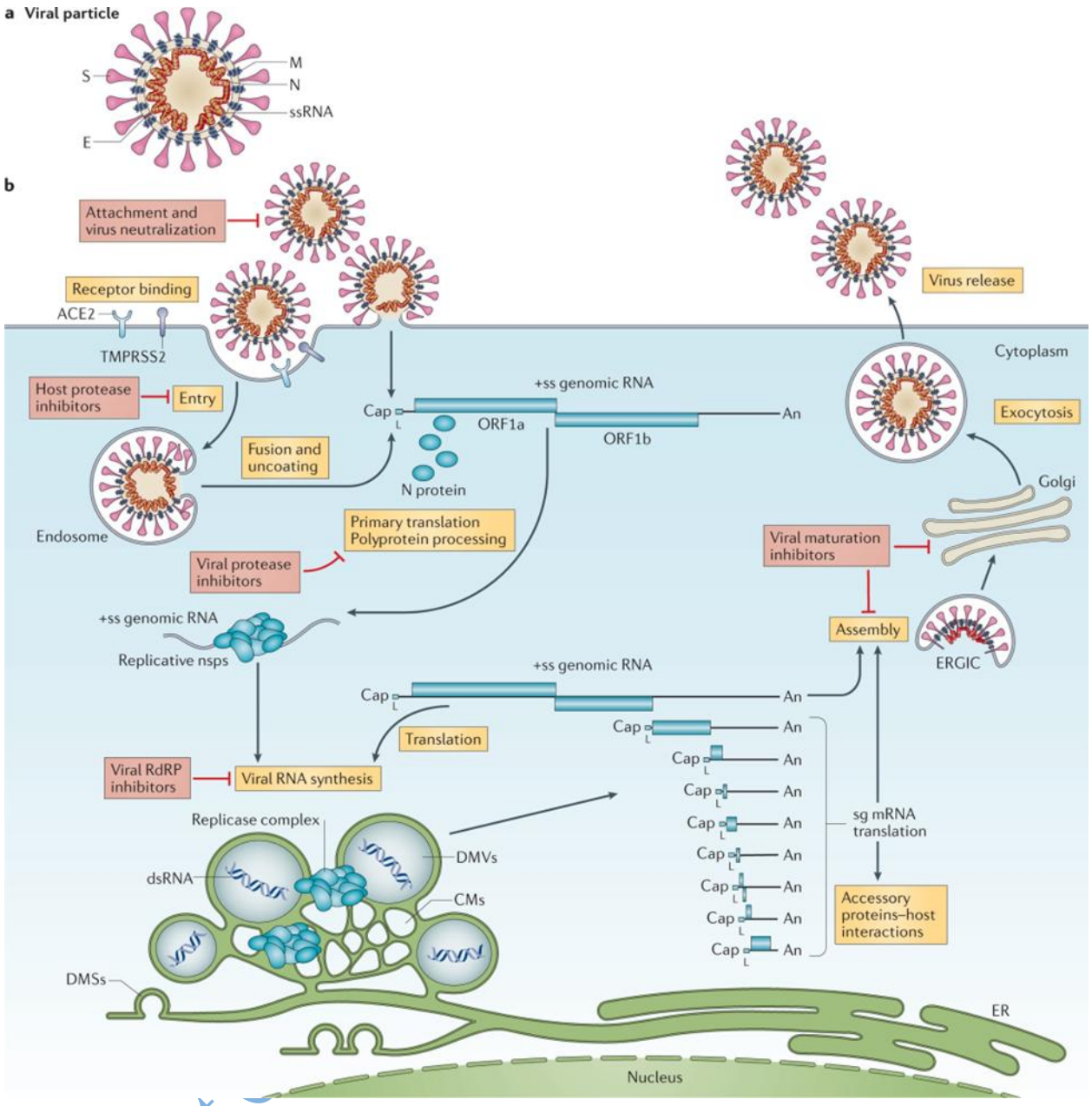

Fig 4.4: Mechanism of viral replication

Source: Nature Reviews Microbiology[31]

The virion of a coronavirus is made up of structural proteins such as spike (S), envelope (E), membrane (M), nucleocapsid (N), and, in the case of some beta coronaviruses, haemagglutinin-esterase. N encapsidates the positive-sense, single-stranded RNA genome (+ssRNA), while M and E ensure its incorporation into the viral particle during the assembly process. S trimers protrude from the viral envelope and serve as receptor specificity for cellular entry receptors. Coronavirus particles bind to cellular attachment factors and specific S interactions with cellular receptors (such as angiotensin-converting enzyme 2 (ACE2), which, in conjunction with host factors (such as the cell surface serine protease TMPRSS2), foster viral uptake and fusion at the cellular or endosomal membrane. Following entry, the incoming genomic RNA is released and uncoated, resulting in the immediate translation of two large open reading frames, ORF1a and ORF1b [32].

The polyproteins pp1a and pp1ab that result are co-translationally and post-translationally processed into the individual non-structural proteins (nsps) that make up the viral replication and transcription complex. Concordant with the expression of nsps, the biogenesis of viral replication organelles made up of characteristic perinuclear double-membrane vesicles (DMVs), small open double-membrane spherules (DMSs) and convoluted membranes (CMs) create a safeguarded micro-environment for viral genomic RNA replication and transcription of subgenomic mRNAs (sg mRNAs) comprising the characteristic nested set of coronavirus mRNAs.Translated structural proteins enter endoplasmic reticulum (ER) membranes and pass through the ER-to-Golgi intermediate compartment (ERGIC), where they interact with N-encapsidated, newly produced genomic RNA, resulting in budding into secretory vesicular compartment lumens. Finally, virions are exocytosed from the infected cell. In red, key steps inhibited by compounds that are currently being validated and represent appealing antiviral targets. An stands for 3′ polyA sequence; cap stands for 5′ cap structure; dsRNA stands for

double-stranded RNA; L stands for leader sequence; and RdRP stands for RNA-dependent RNA polymerase [31].

2.6 Renin-Angiotensin- Aldosterone System Inhibitors and COVID-19

According to the WHO, COVID-19 has affected over 3 million people with thousands of deaths. There has been series of concerns and controversies about how renin-angiotensin-aldosterone system (RAAS) inhibitors affect COVID-19 patients: if there is increase or decrease in the mortality rate due to its use. This has led to several discussions as to whether the use of RAAS inhibitors as treatment options should be reevaluated or not [33]. However, some studies have shown that the rate of increased number of verified COVID-19 cases was not associated with the use of these drugs [1].

Renin-angiotensin-aldosterone system functions in regulating the blood volume and keeping electrolytes in check. It also provides systemic vascular resistance [34]. Angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor type 1 blockers (ARBs) are two medications that suppress the renin-angiotensin-aldosterone system (RAAS) and are frequently used to treat hypertension, heart failure, and coronary heart disease. ACEs and ARBs serve as regulators for RAAS and have been discovered to increase the production of ACE2, a SARS-CoV-2 receptor. This characteristic has brought a lot of attention to the drugs as SARS-CoV-2 may directly interact with the RAAS and jeopardize the regular operation of the RAAS by binding to the angiotensin-converting enzyme 2 (ACE2) as a receptor to facilitate cell fusion and entrance. This phenomenon can be disturbing as studies have shown that patients with SARS-CoV-2 infection most often have hypertension as a concomitant condition [5]. A study in China showed a high prevalence of hypertension among infected individuals [19].

2.7 SARS-CoV-2 and Renin-Angiostensin-Aldosterone System

The critical role played by the renin-angiotensin-aldosterone system in the body cannot be overemphasized. Blood pressure, heart rate, cardiac function, and renal function are all significantly regulated by the renin-angiotensin-aldosterone system [36]. The system is also linked to severe organ injury, particularly acute lung injury, as well as cell proliferation, inflammation, fibrosis, and other side effects. The key chemicals and enzymes that control the function of the RAAS are Ang I, Ang II, Ang 1-7, Ang 1-9, ACE, and ACE2. An effective vasoconstriction and pro-inflammatory activity of Ang II, which is produced from Ang I by the metabolism of ACE, is reliant on Ang II type 1 receptors (AT1Rs), while an opposing impact is produced by targeting AT2Rs [5].

Angiotensinogen (AGT) is broken down into the inactive decapeptide of Ang I by the aspartyl protease renin. The ACE changes Ang I into Ang II's active vasoconstrictor octapeptide of Ang II [37]. When there is a high production of Ang II, inflammation and organ damage can occur [38]. This is due to increased membrane permeability and enhanced epithelial cell death [39]. Additionally, the pro-inflammatory pathway and increased vascular permeability brought on by excessive AT1R activation in the lungs cause lung damage and acute respiratory distress syndrome (ARDS).

ACE2, which is known as a membrane-bound enzyme plays an important role in regulating the lung function and injury on the organ. It creates a balance in the effect of Ang II by synthesizing Ang 1-7 from the metabolism of Ang II. It also converts Ang 1 to Ang 1-9 and subsequently into Ang 1-7 [37]. The latter has anti-inflammatory, antioxidative, and antifibrotic functions which confer a level of protection [38]. By upregulating Ang-(1-7) and depleting Ang II, the use of ACE inhibitors and ARBs may be advantageous in reducing organ damage brought on by COVID-19.

Finally, RAAS dysregulation brought on by higher Ang II and decreased ACE2 can aggravate high blood pressure and trigger an unfavorable inflammatory response. By directly inhibiting Ang II and boosting ACE2, ACE inhibitors and ARBs may have a role in reducing the negative effects.

2.7.1 SARS-CoV-2 infection on ACE2 Expression

SARS-CoV-2 enters cells by ACE2. The virus enters the cells easily by attaching to the membrane bound ACE2 protein, which serves as its functional receptor [40]. However, according to Jung et al. 2020, an aftermath of SARS-CoV2 infection is a reduction in the ACE2 molecules expressed. SARS-CoV-2 has been shown to have the four primary structural proteins spike (S), envelope (E), nucleocapsid (N), and membrane (M). Large membrane-linked glycoproteins called S proteins are essential for viral infection because they connect to the ACE2 receptor on host cells and fuse membranes. The host's vulnerability to SARS-CoV2 infection is determined by the affinity of the S1 subunit, one of the two subunits of the S protein, with the ACE2 receptor. Through binding to ACE2 in a multi-step variation of conformational state, the SARS-CoV-2 is attached to the cell surface, and thus, the commencement of membrane fusion[37].

2.8 Application of RAAS Inhibitors and Associated Risks with COVID 19

Coronary heart disease, hypertension, and diabetes mellitus have been associated as the most prevalent comorbidities among patients with severe COVID-19 illness. These comorbidities are linked to either an excess of Ang II/AT1 or a lack of ACE2 and all have shown improvement with RAAS blocking in circumstances that are chronic. However, in other studies, the persistent usage of renin-angiotensin-aldosterone system (RAAS) inhibitors in people with hypertension has been predicted to worsen disease outcomes. Angiotensin-converting enzyme 2 (ACE2)-receptor is, in fact, specifically recognized by a specified receptor-binding domain of COVID-19 spike [41]. Due to the upregulation of ACE2-receptors

in the cardiopulmonary circulation caused by ACE inhibitors (ACEIs) and angiotensin II receptor blockers (ARBs), patients taking these medications may be more vulnerable to increased severity. In contrast, ACEIs and ARBs may exert protective effects by preventing the progression of pulmonary damage and RAAS hyperactivation because of ACE2-receptor downregulation after COVID-19 infection [33].

Results from several studies disagree with the opinion that the use of ACE inhibitors or ARBs causes an increase in the risk of infection with COVID 19 and morbidity. One such study was conducted by Mehra and colleagues. They carried out a case-control study with patients positive for SARS-CoV 2, but the result showed that there was no correlation between the use of ACE and ARBs and the chances of being infected or having a severe case of the disease [41].

2.8.1 The COVID-19 Spectrum of Illness

Symptoms of post-COVID-19 condition include fatigue, shortness of breath, cognitive dysfunction, and symptoms interfering with daily functioning. These symptoms can remit or relapse over time but persist for at least 2 months and occur 3 months after the initial COVID-19 illness [42].

This seminal cataloguing of symptoms has been supplanted by the urgency to develop a core outcome set (COS) to ensure uniform capture of critically important symptoms for post-COVID-19 clinical research studies. A study using a rigorous Delphi consensus methodology and pre-specified criteria for outcome inclusion, have developed a COS for symptoms of the post-COVID-19 condition that include: fatigue; pain; post-exertion symptoms; work or occupational and study changes; survival; “functioning, symptoms, and conditions” for each of cardiovascular, respiratory, nervous system, cognitive, mental health, and physical outcomes; and recovery [43]. A strength of their study was the recruitment of a diverse and

multidisciplinary investigator group and inclusion of those with lived experience of post-COVID-19 condition and their carriers. The strong foundational work, including the multisource development of an exhaustive a-priori list of candidate post-COVID-19 symptoms, and methodology ensure robust validity of this system.

The determination of both shared and unique symptom distributions across illness severity has important implications for mapping to clinical outcomes, development of a standardized follow-up scheme, and ongoing inter-professional and multidisciplinary longitudinal intervention. Some examples are seen in the Wuhan cohort that was followed for 1 year after recovering from COVID-19 [43]. Almost half of patients across the illness spectrum reported at least one symptom at the 1-year follow-up, although symptom prevalence varied by COVID-19 severity. By contrast, reports of pain and discomfort, anxiety, or depression were similarly represented regardless of illness severity. The entire critically ill group was distinct and reported more dyspnea and had poorer lung function and worse functional outcomes at 1 year than did patients with less severe illness. This finding highlights the dominant contribution of acute respiratory distress syndrome (ARDS) and critical illness sequelae, and the difficulty in discerning what is solely attributable to COVID-19 rather than post-intensive care syndrome[44,45]. The COS will require validation across the spectrum of COVID-19 severity of illness.

2.8.2 The Clinical-Immunological Spectrum of COVID-19

To understand COVID-19 immunopathogenesis, it is important to elucidate what lies at the root of immune response failure occurring in infected individuals resulting at times in deviation of the protective response into a dysfunctional program, leading to cytokine release syndrome (CRS) with severe inflammation and, eventually, a multi-systemic failure. A better understanding of these events would contribute to the design of differential therapeutic approaches, depending on the stage of the disease, and to the delineation of prognostic, and

predictive biomarkers. Unfortunately, there are no studies on the immune response in infected asymptomatic individuals, which would allow a better characterization of the protective immune response as it occurs under the natural conditions of the infection process. Another aspect to be explored is the effect of previous exposure to other less virulent coronaviruses that may have cross-reactivity with more virulent ones. Additionally, most of the studies have been done using blood samples, which do not necessarily correlate with the events going on in the affected tissues. Fortunately, several studies on bronchoalveolar lavage cells were published recently, as will be discussed below [44].

From an immunological point of view, the wide clinical spectrum of COVID-19 allows us to postulate different hypotheses, some of them which have already been proven, the remaining requiring more information and longer follow-up observation of recovered patients. Figure 1A shows diverse outcomes during COVID-19 and allows for an analysis of the immune response at each clinical stage. However, it must be noted that the immune response is conditioned by epidemiological variables, such as intensity and duration of exposure to the virus and possible variations in viral virulence and, on the host side, genetic susceptibility/resistance, and health conditions at the time of exposure. The latter includes, among other variables, age and the existence of comorbidities that may directly affect the immune system [45].

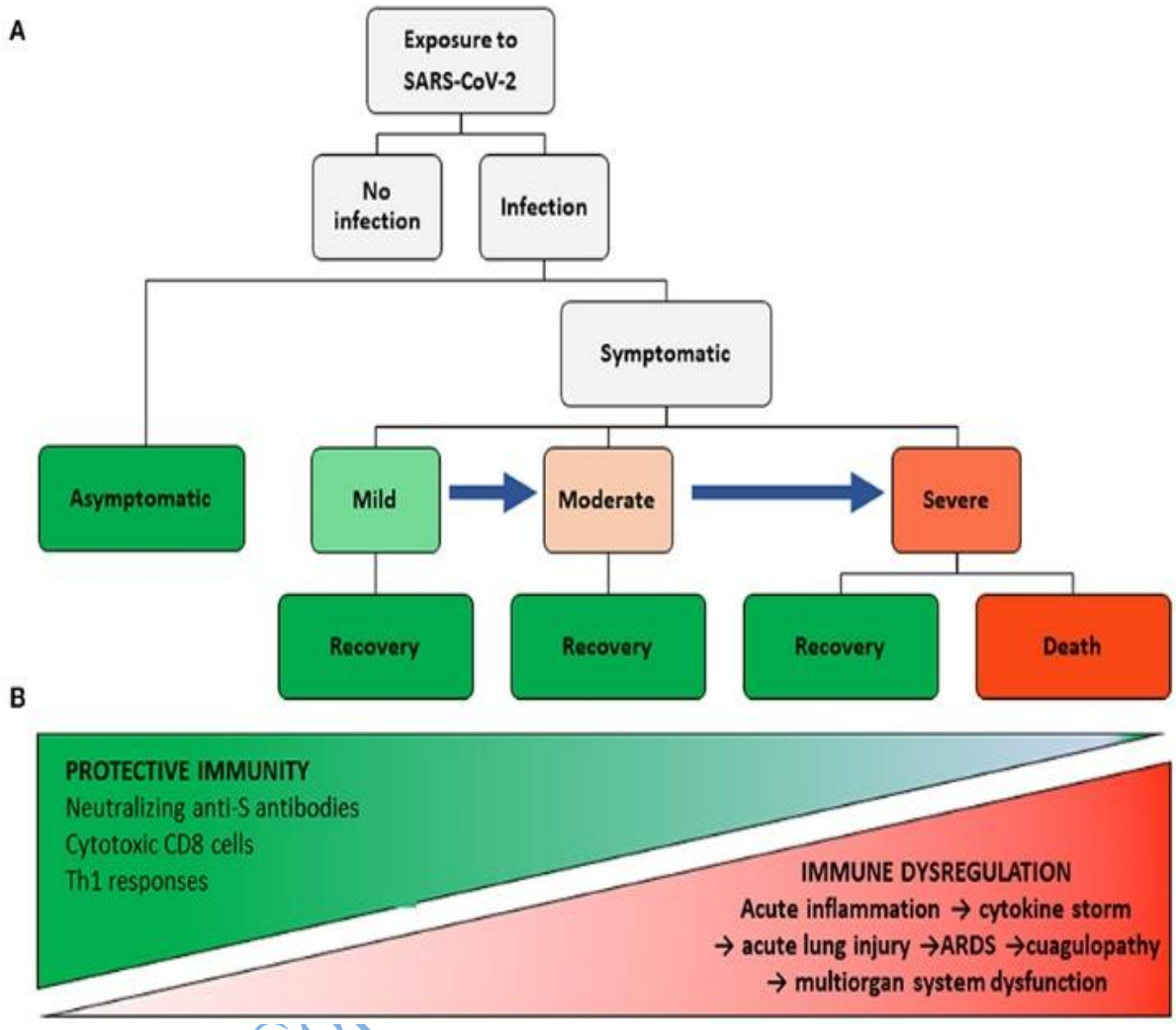

Figure 2.5. COVID-19 Clinical and immunological spectra. (A) Clinical stages of COVID-19. (B) Protective immunity and inflammatory spectra.

Source: The Lancet Respiratory Medicine, 2020[33]

Despite its high infectivity, not everyone exposed to SARS-CoV-2 becomes infected [46]. The reasons for such resistance are still unknown. It is possible that a small, occasional inoculum does not reach the lower respiratory tract, where susceptible target cells are found. Nevertheless, yet unidentified genetic conditions may also explain this resistance. On this regard, no association of SARS with ACE2 polymorphisms was found [47].

2.9 Disease Complications

2.9.1 Cardiovascular Complications Associated with COVID-19 Infection.

Acute Myocardial Infarction (AMI)

Due to extensive inflammation and hypercoagulability, the risk of acute myocardial infarction (AMI) is likely present in patients with COVID-19 [48]. The treatment of AMI is controversial in COVID-19 patients. In patients diagnosed with an ST elevation myocardial infarction (STEMI) and COVID-19, the American College of Cardiology (ACC) states that while fibrinolysis may be considered in those with "low risk STEMI", defined by inferior STEMI with no right ventricular involvement or lateral AMI without hemodynamic compromise, percutaneous coronary intervention (PCI) is more commonly performed at most institutions and remains the treatment of choice [49]. If PCI is pursued, staff should don appropriate personal protective equipment (PPE), and a full decontamination of the catheterization laboratory should be performed following the procedure. For suspected COVID-19 in the setting of NSTEMI, diagnostic testing prior to catheterization is recommended; the ACC notes that, in properly selected patients with confirmed COVID-19, conservative therapy may be sufficient. Patients who are hemodynamically unstable in the setting of NSTEMI should be managed similarly to those with STEMI [49].

2.10 Long-term Complications of COVID-19

2.10.1 Diabetes Complications and Sequelae

Preexisting diabetes mellitus has been associated with worse COVID-19 outcomes. At the same time, COVID-19 has been associated with new-onset hyperglycemia and acute decompensation of diabetes, including diabetic ketoacidosis in both patients with type 1 and type 2 diabetes [50]. Besides iatrogenic hyperglycemia from steroid use, proposed mechanisms for hyperglycemia following infection include insulin resistance because of the inflammatory state, and insulin secretory deficits from impaired β cells—either due to direct viral damage or indirect effects [51]. It is unclear how many patients who are newly diagnosed with diabetes after COVID-19 already had unrecognized diabetes before infection, and simply had their diabetes unmasked or exacerbated. It is also unclear whether new-onset diabetes following hospitalization for COVID-19 is permanent. Therefore, the global CoviDiab Registry was created to further study the relationship between COVID-19 and diabetes, and to better characterize the duration of post-COVID-19 diabetes [52].

2.9.2 Dermatological Complications and Sequelae

Of the reported cutaneous manifestations of COVID-19 infection, a literature review found that 36.1% of 72 documented patients across 18 studies reported maculopapular exanthem (morbilliform) as the most common cutaneous manifestation of COVID-19, followed by papulovesicular rash (34.7%), urticaria (9.7%), and painful red acral purple papules (15.3%), with 19.4% of these manifestations being in the hands and feet [53]. Another international study of 2,560 patients found that pernio-like lesions were the most common cutaneous manifestation (51.5%), with the latency time between upper-respiratory infections and cutaneous findings being 1.5 days in children versus 7.9 days in adults [51]. In the Chinese post-acute COVID-19 study of hospitalized patients, only 47 of 1,655 patients (3%) reported skin rashes 6 months after infection onset [43]; instead, hair loss was a far more commonly reported

symptom for patients' months after COVID-19 infection reported in 24 of 120 patients (20.0%) as a post discharge symptom 110 days after hospital discharge [54]. Still, other rarer presentations have been reported in case reports, suggesting that manifestations in different patients may be different despite being infected with the same virus [55]. Although it has been suggested that vesicular rashes may be indicative of an initial diagnosis of COVID-19 and vascular rashes may be indicative of disease prognosis, the precise use of these symptoms in this fashion has not yet been validated and should be a subject of future prospective studies [56].

2.11 Vaccination

Vaccination is the administration of a vaccine to help the immune system develop immunity from a disease[57]. Vaccines contain a microorganism or virus in a weakened, live or killed state, or proteins or toxins from the organism. In stimulating the body's adaptive immunity, they help prevent sickness from an infectious disease. When a sufficiently large percentage of a population has been vaccinated, herd immunity results. Herd immunity protects those who may be immunocompromised and cannot get a vaccine because even a weakened version would harm them[58]. The effectiveness of vaccination has been widely studied and verified. Vaccination is the most effective method of preventing infectious diseases; widespread immunity due to vaccination is largely responsible for the worldwide eradication of smallpox and the elimination of diseases such as polio and tetanus from much of the world. However, some diseases, such as measles outbreaks in America, have seen rising cases due to relatively low vaccination rates in the 2010s – attributed, in part, to vaccine hesitancy. According to the World Health Organization, vaccination prevents 3.5–5 million deaths per year[59].

Vaccines reduce risks of getting a disease by working with your body's natural defences to build protection[60]. When one get a vaccine, the immune system responds. There are vaccines to prevent more than 20 life-threatening diseases, helping people of all ages live longer,

healthier lives. Immunization currently prevents 3.5-5 million deaths every year from diseases like diphtheria, tetanus, pertussis, influenza and measles. Vaccines are also critical to the prevention and control of infectious disease outbreaks. They underpin global health security and will be a vital tool in the battle against antimicrobial resistance[61].

Yet despite tremendous progress, vaccination coverage has plateaued in recent years and dropped since 2020. The COVID-19 pandemic and associated disruptions over the past two year have strained health systems, with 25 million children missing out on vaccination in 2021, 6 million more than in 2019 and the highest number since 2009. By the end of 2021, nearly all countries had introduced COVID-19 vaccination, and by early 2022 one billion doses of COVID-19 vaccine had been delivered through COVAX[62].

2.11.1 SARS-CoV-2 Vaccination

COVID-19 vaccines have been instrumental in curbing the intensity of this pandemic and decreasing the morbidity and mortality of this disease[63]. COVID-19-associated hospitalizations and emergency/urgent care evaluations significantly improved with these vaccines. A study from Israel stated that two doses of BNT162b2 were highly effective in preventing symptomatic and asymptomatic SARS-CoV-2 infections, as well as COVID-19-related hospitalizations, severe disease, and death[64]. A study from the United States evaluated the efficacy of BNT162b2 and mRNA-1273 vaccines in preventing severe illness for individuals at the highest risk of severe COVID-19 illness (those with advanced age and underlying comorbidities) by comparing their outcomes to unvaccinated individuals. The study stated that vaccine effectiveness 7 or more days after the second dose of the vaccine was 69% in preventing infection and 86% against severe disease/death. They concluded that in an elderly population with a high-comorbidity burden, the vaccine effectiveness was lower than previously projected; however, the efficacy against death was high[65].

2.11.2 Mechanism of Action

The mRNA class of vaccines' rapid evolution and advancements are from the mRNA capabilities to imitate the antigen structure and expression comparable to those occurring during the SARS-CoV-2 infection. The mRNA class of the vaccine does not pose a risk of infection or insertional mutagenesis. The mRNA eludes the anti-vector immunity permitting repeated vaccinations. The mRNA's ability to produce an undesired immune response within the body can be reduced, and modifications can be arranged to improve the mRNA vaccines' determination[66].

BNT162b2

BNT162b2 is a nucleoside-modified messenger RNA (mRNA) expressed in lipid nanoparticles (LNP), encoding the spike (S) protein for the SARS-CoV-2 virus - the main site for neutralizing antibodies. The lipid particles allow the transfer of the RNA into host cells, resulting in the SARS-CoV-2 S antigens' expression. The immunogenicity and antibody response to SARS-CoV-2 S antigens further confers protection against COVID-19[67].

mRNA-1273

The mRNA-1273 vaccine is comprised of a lipid nanoparticle (LNP) encapsulating nucleoside-modified messenger RNA (mRNA), encoding the perfusion stabilized spike (S) protein of the SARS-CoV-2 virus and an S1-S2 cleavage site, which includes a transmembrane component. The S-2P antigen present on its surface allows entrance into the host cell. This further transfers the RNA into host cells, resulting in the expression of the SARS-CoV-2 S antigens. The immunogenicity and antibody response to SARS-CoV-2 S antigens also confers protection against COVID-19[68].

Ad26.COV2.S

The Ad26.COV2.S exerts its effects by expressing the SARS-CoV-2 S protein to human cells creating an immune response to the SARS-CoV-2 antigen, conferring immunity without dispersal of the virus. Different from the mRNA vaccines (BNT162b2 and mRNA-1273), the Ad26.COV2.S is a recombinant viral vector vaccine. The Ad26.COV2.S recombinant viral vector vaccine bears DNA which forms the S protein of the SARS-CoV-2 virus, once injected into host cells, provide considerable amounts of the S protein antigen. The immune response to the S protein confers immunity by T-cells and B-cell stimulating antibodies to the S protein, similar to SARS-CoV-2 infection[69].

NVX-CoV2373

This vaccine contains recombinant spike (rS) protein nanoparticles and Matrix-M adjuvant proteins. The rS protein is produced using recombinant DNA technology. Intramuscular injection of the full-length rS protein elicits an immune response to the rS protein, which protects against COVID-19 illness[70].

2.11.3 Different types of SARS-CoV-2 Vaccinations

There are four categories of vaccines in clinical trials: whole virus, protein subunit, viral vector and nucleic acid (RNA and DNA). Some of them try to smuggle the antigen into the body, others use the body's own cells to make the viral antigen.

Whole Virus

Many conventional vaccines use whole viruses to trigger an immune response. There are two main approaches. Live attenuated vaccines use a weakened form of the virus that can still replicate without causing illness[71]. Inactivated vaccines use viruses whose genetic material has been destroyed so they cannot replicate, but can still trigger an immune response. Both types use well-established technology and pathways for regulatory approval, but live attenuated ones may risk causing disease in people with weak immune systems and often

require careful cold storage, making their use more challenging in low-resource countries. Inactivated virus vaccines can be given to people with compromised immune systems but might also need cold storage[72].

Protein Subunit

Subunit vaccines use pieces of the pathogen often fragments of protein to trigger an immune response. Doing so minimises the risk of side effects, but it also means the immune response may be weaker. This is why they often require adjuvants, to help boost the immune response. An example of an existing subunit vaccine is the hepatitis B vaccine. Subunit vaccines include only the parts of a virus that best stimulate your immune system. This type of COVID-19 vaccine contains harmless S proteins. Once the immune system recognizes the S proteins, it creates antibodies and defensive white blood cells. If later become infected with the COVID-19 virus, the antibodies will fight the virus[73].

Viral Vector

Viral vector vaccines also work by giving cells genetic instructions to produce antigens. But they differ from nucleic acid vaccines in that they use a harmless virus, different from the one the vaccine is targeting, to deliver these instructions into the cell. One type of virus that has often been used as a vector is adenovirus, which causes the common cold. As with nucleic acid vaccines, our own cellular machinery is hijacked to produce the antigen from those instructions, in order to trigger an immune response. Viral vector vaccines can mimic natural viral infection and should therefore trigger a strong immune response. However, since there is a chance that many people may have already been exposed to the viruses being used as vectors, some may be immune to it, making the vaccine less effective[74].

Nucleic Acid

Nucleic acid vaccines use genetic material from a disease-causing virus to trigger protective immunity against it. Nucleic acid vaccines use genetic material from a disease-causing virus

or bacterium (a pathogen) to stimulate an immune response against it. Depending on the vaccine, the genetic material could be DNA or RNA; in both cases it provides the instructions for making a specific protein from the pathogen, which the immune system will recognise as foreign (an antigen). Once inserted into host cells, this genetic material is read by the cell's own protein-making machinery and used to manufacture antigens, which then trigger an immune response[75].

2.12 Renal System

The renal system consists of the kidney, ureters, and the urethra. The overall function of the system filters approximately 200 liters of fluid a day from renal blood flow which allows for toxins, metabolic waste products, and excess ion to be excreted while keeping essential substances in the blood[76]. The kidney regulates plasma osmolarity by modulating the amount of water, solutes, and electrolytes in the blood. It ensures long term acid-base balance and also produces erythropoietin which stimulates the production of red blood cell. It also produces renin for blood pressure regulation and carries out the conversion of vitamin D to its active form.

In many respects the human excretory, or urinary, system resembles those of other mammalian species, but it has its own unique structural and functional characteristics. Theterm excretoryand *urinary* emphasize the elimination function of the system. The kidneys, however, both secrete and actively retain within the body certain substances that are as critical to survival as those that are eliminated[77].

The system contains two kidneys, which control the electrolyte composition of the blood and eliminate dissolved waste products and excess amounts of other substances from the blood; the latter substances are excreted in the urine, which passes from the kidneys to the bladder by way of two thin muscular tubes called the ureters. The bladder is a sac that holds the urine until it is eliminated through the urethra[78].

Three different sets of kidneys develop consecutively from the urogenital ridges, and the last set persists to become the adult kidney. The first renal tubular system is called the pronephros. Pronephros develops during the fourth week of embryonic development but quickly degenerates as mesonephros appears[79]. Mesonephric kidney degenerates as the metanephros develops through its remnant are incorporated into the male reproductive system. The metanephros begins its development around the fifth week of embryonic development as ureteric buds. As the ureteric buds develop, it induces the formation nephrons. The distal ends of the ureteric buds develop into the renal pelvis, calyces, and collecting ducts as the proximal aspect of the ureteric buds develop into ureters. A structure called cloaca develops to form the rectum, anal canal, and urogenital sinus. The urogenital sinus then forms into the urinary bladder and the urethra. By the third month of fetal development, metanephric kidney is able to excrete urine into the amniotic fluid[80].

2.12.1 Mechanism of Action

Glomerular Filtration

Glomerular filtration is the initial process in urine production. It is a passive process in which hydrostatic pressure pushes fluid and solute through a membrane with no energy requirement[81]. The filtration membrane has three layers: fenestrated endothelium of the glomerular capillaries which allow blood components accept the cells to pass through; basement membrane, which is a negatively charged physical barrier that prevents proteins from permeating; and foot processes of podocytes of the glomerular capsule that creates more selective filtration. The outward and inward force from the capillaries determines how much water and solute crosses the filtration membrane[82]. Hydrostatic pressure from the glomerular capillaries is the major filtration force with a pressure of 55mmHg. The other potential filtration force is the capsular space colloid osmotic pressure, but it is zero because proteins are not usually present within the capsular space. Then the capsular space hydrostatic

pressure and the colloid osmotic pressure in glomerular capillaries negate the filtration force from the hydrostatic pressure in the glomerular capillaries, creating a net filtration pressure which plays a big role in the glomerular filtration rate (GFR)[83].

GFR is the volume of fluid filtered in a minute, and it depends on the net filtration pressure, the total available surface area for filtration, and filtration membrane permeability. The normal GFR is between 120 to 125ml/min [84]. It is regulated intrinsically and extrinsically to maintain the GFR. The intrinsic control function by adjusting its own resistance to blood flow via a myogenic mechanism and a tubuloglomerular feedback mechanism. The myogenic mechanism maintains the GFR by constricting the afferent arteriole when the vascular smooth muscle stretches due to high blood pressure. It dilates the vascular smooth muscle when pressure is low within the afferent arteriole allowing more blood to flow through[85]. Then the tubuloglomerular feedback mechanism function to maintain the GFR by sensing the amount of NaCl within the tubule. Macula densa cells sense NaCl around the ascending limb of the nephron loop.When blood pressure is high, the GFR will also be high; this decreases the time needed for sodium reabsorption, and therefore sodium concentration is high in the tubule. The macula densa cell senses it and releases the vasoconstrictor chemicals which constricts the afferent arteriole and reduces blood flow. Then when the pressure is low, Na gets reabsorbed more causing its concentration in the tubule to be low, and macula densa do not release vasoconstricting molecules[86].

The extrinsic control maintains the GFR and also maintains the systemic blood pressure via the sympathetic nervous system and the renin-angiotensin-aldosterone mechanism. When the volume of fluid in the extracellular decreases excessively, norepinephrine and epinephrine get released and causes vasoconstriction leading to a decrease in blood flow to the kidney and the level of GFR[87]. Also, the renin-angiotensin-aldosterone axis gets activated by three means when the blood pressure drops. The first is the activation of the beta-1 adrenergic receptor,

which causes the release of renin from the granular cells of the kidney. The second mechanism is the macula densa cells which senses low NaCl concentration during decreased blood flow to the kidney and trigger the granular cells to release renin. The third mechanism is the stretch receptor around the granular cells senses decreased tension during decreased blood flow to the kidney and also trigger the release of renin, therefore, regulating the glomerular filtration[88].

Tubular Reabsorption

The four different tubular segments each have unique absorptive properties. The first is the proximal convoluted tubule (PCT). The PCT cells have the most absorptive capability[89]. In the normal circumstance, the PCT reabsorbs all the glucose and amino acids as well as 65% of Na and water. The PCT reabsorb sodium ions by primary active transport via a basolateral Na-K pump. It reabsorbs glucose, amino acids, and vitamins through secondary active transport with Na and an electrochemical gradient drives passive paracellular diffusion. The PCT reabsorbs water by osmosis that is driven by solute reabsorption. It also reabsorbs lipid-soluble solutes via passive diffusion driven by the concentration gradient created by reabsorption of water. Reabsorption of urea occurs in the PCT as well by passive paracellular diffusion driven by a chemical gradient[90].

From the PCT, the non-reabsorbed filtrates move on to the nephron loop. The nephron loop functionally divides into a descending and an ascending limb. The descending limb functions to reabsorb water via osmosis[91]. This process is possible due to the abundance of aquaporins. Solutes do not get reabsorbed in this region. However, in the ascending limb, Na moves passively down its concentration gradient in the thin segment of the ascending limb, and also sodium, potassium, and chlorides get reabsorbed together through a symporter in the thick segment of the ascending limb[92]. The presence of Na-K ATPase in the basolateral membrane keeps this symporter functional by creating an ionic gradient. There is also the reabsorption

of the calcium and magnesium ions in the ascending limb via passive paracellular diffusion driven by the electrochemical gradient. No water reabsorption in the ascending limb[93].

The next tubular segment for reabsorption is the distal convoluted tubule (DCT). There is a primary active sodium transport at the basolateral membrane and secondary active transport at the apical membrane through Na-Cl symporter and channels. This process is aldosterone regulated at the distal portion. There is also calcium reabsorption via passive uptake controlled by the parathyroid hormone. Aldosterone targets the cells of the distal portion of the DCT causing synthesis and retention of apical Na and K channel as well as the synthesis of Na-K ATPase[94].

Right after the DCT, there is a collecting tubule where the final stage of reabsorption occurs. The reabsorptions that occur here include primary active sodium transport at basolateral membrane; secondary active transport at apical membrane via Na-Cl symporter and channels with aldosterone regulation; passive calcium uptake via PTH-modulated channels in the apical membrane; and primary and secondary active transport in the basolateral membrane[95].

Tubular Secretion

Tubular secretion function is to dispose of substances such as drugs and metabolites that bind to plasma protein. Tubular secretion also functions to eliminate undesirable substances that were reabsorbed passively such as urea and uric acids[96]. Elimination of excess potassium via aldosterone hormone regulation at collecting duct and distal DCT are part of tubular secretion function. There is an elimination of hydrogen ion when the blood pH drops below the normal range. Then when the blood pH increases above the normal range, reabsorption of chloride ions occurs as bicarbonic acid gets excreted. The secretion of creatinine, ammonia, and many other organic acids and basics occur[97].

Storage of Urine

Once the production of urine is complete, it travels through a structure called ureter for urine storage in the bladder[98]. There are two ureters in a human body; one on each side; left and right. They are slender tubes with three-layered walls: the mucosa that contains a transitional epithelial tissue; muscularis that is composed of the internal longitudinal layer and the external circular layer; and adventitia that is a fibrous connective tissue that covers the ureter's external surface. As urine make its way to the ureters, the stretching of the ureter's smooth muscle results in peristaltic contractile waves that help move the urine into the bladder[99]. The oblique insertion of the ureter at the posterior bladder wall prevents backflow of urine. Once the urine is in the bladder, the bladder's unique anatomy allows for efficient storage of urine[99].

The bladder is essentially a muscular sac with three layers. Its three layers are similar to the ureter except that the muscular layer has muscle fibers organized in inner and outer longitudinal layers and a middle circular layer[100]. The muscular layer is also known as the detrusor muscle. The distensibility of the bladder allows it to hold a maximum capacity of up to 1000ml, though normal functional capacity is 300 to 400mL. The bladder has three openings at the smooth triangular region of the bladder; this is called the trigone. Two of the openings are where the distal portions of the ureters insert, and the other opening is the orifice for the urethra[101].

The urethra is a thin-walled muscular tube that functions to drain urine out of the bladder. Its mucosa lining consists of mostly pseudostratified columnar epithelium through the proximal portion has transitional epithelial tissue[102]. The thickening of the detrusor muscle at the bladder-urethra junction forms the internal urethral sphincter which has an autonomic nervous system control. The urethra has an additional function for males as it transports semen. In males, the urethra is approximately 22.3 cm long with three regions which include

the prostatic urethra, membranous urethra, and the spongy urethra. Females, on the other hand, has a urethra that is approximately 3.8 to 5.1 cm long with an external urethral orifice that lies anterior to the vaginal opening and posterior to the clitoris[103].

2.12.2 Micturition Process

Micturition process entails contraction of the detrusor muscle and relaxation of the internal and external urethral sphincter[104]. The process is slightly different based on age. Children younger than three years old have the micturition process coordinated by the spinal reflex. It starts with urine accumulation in the bladder that stretches the detrusor muscle causing activation of stretch receptors. The stretch sensation is carried by the visceral afferent to the sacral region of the spinal cord where it synapses with the interneuron that excites the parasympathetic neurons and inhibits the sympathetic neurons[105]. The visceral afferent impulse concurrently decreases the firing of the somatic efferent that normally keeps the external urethral sphincter closed allowing reflexive urine output. However, after the age of 3, there is an override of reflexive urination where there is conscious control of the external urethral sphincter[106]. High bladder volume activates the pontine micturition center which activates the parasympathetic nervous system and inhibits the sympathetic nervous system as well as triggers awareness of a full bladder; consequently leading to relaxation of the internal sphincter and a choice to relax the external urethral sphincter once ready to void. Low bladder volume activates the pontine storage center which activates the sympathetic nervous system and inhibits the parasympathetic nervous system cumulatively allowing the accumulation of urine in the bladder[107].

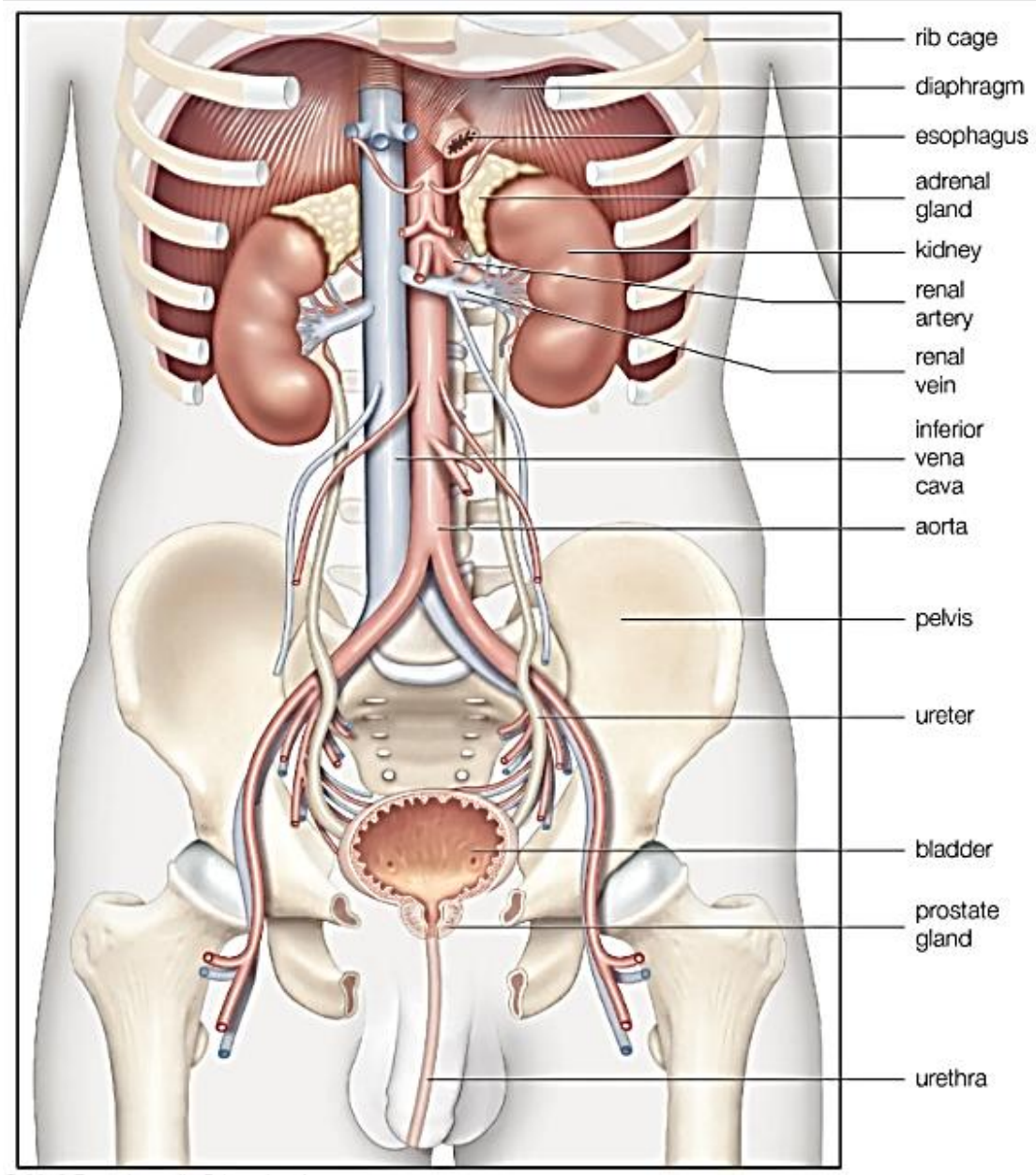

Figure 2.6 The Kidney Function Markers

Source:Pharmaceuticals, 2023[104]

The kidney performs many excretory and regulatory functions necessary to sustain life. Under normal conditions, the kidney not only functions to maintain the constancy of the extracellular environment by excretion of the waste products of metabolism and the adjustment of urinary water and electrolyte excretion, but also is intricately involved in the regulation of blood pressure, red blood cell production, and bone mineral metabolism[108]. With this in mind, it is not surprising that a variety of diverse biological markers are employed in clinical practice to monitor the physiologic status of the kidney. Many of the markers in use presently have been employed for decades, although there has been a surge in biomarker discovery in recent years that promises to augment assessment of kidney function and injury.

The markers of renal function test assess the normal functioning of kidneys. These markers may be radioactive and non-radioactive. They indicate the glomerular filtration rate, concentrating and diluting capacity of kidneys (tubular function). If there is an increase or decrease in the valves of these markers it indicates dysfunction of kidney[109].

Biochemical markers play an important role in accurate diagnosis and also for assessing risk and adopting therapy that improves clinical outcome. Over decades of research and utilization of biomarkers has evolved substantially[110]. National Institute of Health (NIH) 2001 defined a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological, pathologic processes, or pharmacologic responses to a therapeutic intervention. As markers of renal function creatinine, urea, uric acid and electrolytes are for routine analysis whereas several studies have confirmed and consolidated the usefulness of markers such as cystatin C, β-Trace Protein[111].

2.12.3 Renal Function Markers

a. Creatinine

Creatinine is a breakdown product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body depending on muscle mass. Creatinine is a commonly used as measure of kidney function.The normal creatinine clearence test valve is 110-150ml/min in male and in female it is 100-130ml/min[112]. The National Kidney Disease Education Program recommends calculating glomerular filtration rate from serum creatinine concentration. The creatinine clearance test is used to monitor the progression of renal disease. The diagnosis of renal failure is usually suspected when serum creatinine is greater than the upper limit of the "normal" interval. In chronic renal failure and uremia, an eventual reduction occurs in the excretion of creatinine by both the glomeruli and the tubules[113]. Creatinine values may alter as its generation may not be simply a product of muscle mass but influenced by muscle function, muscle composition, activity, diet and health status. The increased tubular secretion of creatinine in some patients with kidney dysfunction could give false negative value. The elevated values are also seen in muscular dystrophy paralysis, anemia, leukemia and hyperthyroidism. The decreased values are noticed with glomerulonephritis, congestive heart failure, acute tubular necrosis, shock, polycystic kidney disease, and dehydration[114].

b. Urea

Urea is major nitrogenous end product of protein and amino acid catabolism, produced by liver and distributed throughout intracellular and extracellular fluid. In kidneys urea is filtered out of blood by glomerulli and is partially being reabsorbed with water[115]. The most frequently determined clinical indices for estimating renal function depends upon concentration of urea in the serum. It is useful in differential diagnosis of acute renal failure and pre renal condition where blood urea nitrogen–creatinine ratio is increased[116]. Urea clearance is a poor indicator of glomerular filtration rate as its overproduction rate depends

on several non renal factors, including diet and urea cycle enzymes. Increased blood urea nitrogen (BUN) is seen associated with kidney disease or failure, blockage of the urinary tract by a kidney stone, congestive heart failure, dehydration, fever, shock and bleeding in the digestive tract. The high BUN levels can sometimes occur during late pregnancy or result from eating large amounts of protein-rich foods. If the BUN level is higher than 100 mg/dL it points to severe kidney damage whereas decreased BUN is observed in fluid excess. Low levels are also seen in trauma, surgery, opioids, malnutrition, and anabolic steroid use[117].

c. Cystatin C

The protease inhibitor Cystatin C is a non-glycosylated low molecular weight protein. Cystatin C has been proposed to be a marker as it is produced by all nucleated cells at a constant rate and is freely filtrated by the glomeruli and completely catabolized in the proximal tubules[118]. The concentration of serum Cystatin C is mainly determined by glomerular filtration, which makes Cystatin C an endogenous marker of glomerular filtration rate. In a Meta analysis study by Dharnidharka et al found Cystatin C was superior to serum creatinine as a marker of glomerular filtration rate. Other studies have shown similar results when compared with other markers such as a 1-microglobulin and β 2-microglobulin[119]. Cystatin C was found to be an effective marker for glomerular filtration rate in patients with cirrhosis following liver transplantation. Cystatin C has been found more useful for detecting early renal impairment in both type 1 and type 2 diabetic patients. Moreover, cystatin C was also found to be associated with mild kidney dysfunction with increased risk for cardiovascular events, peripheral arterial disease and heart failure[120].

d. β -Trace Protein (BTP)

This protein is filtered at glomerulus and then reabsorbed in proximal tubule or excreted in urine and hence have potential to meet the criteria for use as a marker of glomerular filtration rate. β-Trace Protein is a low-molecular weight glycoprotein belonging to the lipocalin

protein family with 168 amino acids and a molecular weight of 23000–29000, depending on the degree of glycosylation. It has been reported to be a better indicator of reduced glomerular filtration rate than serum creatinine. Serum β -Trace Protein has been found to be elevated in patients with renal diseases. However, when compared, Cystatin C is still a better indicator than Serum β-Trace Protein[121].

e. Inulin

Fructose polymer inulin (MW 5kDa) satisfies the criteria as an ideal marker of glomerular filtration rate. Rapid measurement of glomerular filtration rate by an inulin single-bolus technique would be practically useful[122].

f. Iohexol

A new technique of measuring iohexol clearance using timed dried capillary blood spots was shown. Blood spot iohexol clearance showed potential in estimating glomerular filtration rate accurately in large-scale epidemiological studies especially among individuals without established chronic kidney disease. Plasma clearance after single injection of iohexol gives a good estimate of glomerular filtration rate and is advantageous for the patients and clinicians. Iohexol clearance is also used to estimate residual renal function in hemodialysis patients[123].

g. Radioactive Markers

In recent decade radioisotopes markers have been used to measure glomerular filtration rate. Some of them to mention are 125iodine (I)-iothalamate, 51CrEDTA ethylenediamine tetra acetic acid, 99mTc-DTPA (diethylene triamine penta acetic acid) and 99mTc mercapto acetyl triglycine. Renal 125iodine (I)-iothalamate clearance, is a simple and accurate test after a single subcutaneous injection, to measure glomerular filtration rate in adults[124]. The same author found renal clearance of 125 iodine (I)-iothalamate was reproducible, simple, and practical in healthy children and those with mild and advanced renal disease. In one of the study the mean renal extraction of Cystatin C was equal to the mean renal extraction of 125

iodine (I)-iothalamate in hypertensive patients, suggesting tubular secretion of Cystatin C[125]. It was possible to get an accurate determination of 5lCr-EDTA clearance from a single-plasma sample in adults by applying the mean sojourn time-based approach previously shown to be very precise for determination of 99mTc-DTPA single-sample clearance. 5lCr EDTA-glomerular filtration rate is suggested for systemic lupus erythematosus patient with suspected renal involvement even when the serum creatinine concentration and creatinine clearance are normal. The limitation of this marker is that glomerular filtration rate measured by 5lCr EDTA can be overestimated in patients with severe oedema[126].

h. Proteinuria

Clinically the appearance of significant amount of protein in urine is one of the earliest sign of almost all renal diseases. Estimation of proteinuria helps in differentiating between tubulointerstitial and glomerular diseases and also to follow the progress of renal disease and to assess the response to therapy[127]. Normally excretion in most healthy adults is between 20-150 mg of protein in urine over 24 hrs. Proteinuria more than 3.5 gm/day is taken to be diagnostic of nephrotic syndrome. Panels of protein measurement including albumin, α 2-macroglobulin, IgG and α 2-microglobulin have been employed in differential diagnosis of prerenal and postrenal disease. It has been recommended the use of the protein/creatinine ratio as an Index of Quantitative Proteinuria in 24-hour urine collection. The prevalence of kidney diseases in people with diabetes was found to have proteinuria. The use of the clearance of haptoglobin, in particular provided valuable diagnostic information in cases in which the routine methods gave borderline values for the index of proteinuria[128]. During pregnancy proteinuria assay in 24-hour urine sample is performed. One of the investigations for proteinuria is semi-quantitative dipstick urinalysis as this method is relatively low cost and easily performed. In pregnancy automated dipstick urinalysis is a more accurate screening test for the detection of proteinuria than visual testing. The finding of dipstick

proteinuria should be confirmed by either a 24-hour urine collection or a protein-creatinine ratio[129].

i. Markers of tubular function

Tubular function tests involve evaluation of functions of the proximal tubule (i.e. tubular handling of sodium, glucose, phosphate, calcium, bicarbonate and amino acids) and distal tubule (urinary acidification and concentration). A study earlier done assessed the renal proximal tubular function in neonates by measuring urinary β 2-microglobulin concentrations[130]. While another study also showed that in sick neonates the urinary β 2-microglobulin and N-acetyl-β-D-glucosaminidase were the early markers of renal tubular dysfunction. They concluded that the elevated levels of urinary β 2-microglobulin and N-acetyl-β-D-glucosaminidase in neonates born with meconium-stained amniotic fluid indicated the existence of tubular dysfunction, probably due to prenatal distress[131].

j. Concentration and Dilution methods

Serum osmolality was measured directly using osmometry, or estimated based on the direct measurement of the concentrations of the osmotically active substances (i.e. sodium, glucose, blood urea nitrogen, and ethanol) [132]. The difference between the measured osmolality and the calculated molarity is referred to as the osmole gap. Laloë et al observed severe hyponatraemia in some of the patients by measuring urine osmolality and urine sodium. According to Jeff MS there are some genes which are involved in urine concentration which may encode solute-transport proteins and the vasopressin receptors. These molecular mechanisms show the reduction in urine-concentrating ability with aging that predicts various changes in kidney function. While another work showed that aquaporin-1 has a physiologic role in renal function and is also essential for maximal urinary concentrating ability. In a complete deficiency of Aquaporin-1 there is defective urine concentrating ability[133].

k. Electrolyte

Electrolyte panel is frequently used to screen for an electrolyte or acid-base imbalance and to monitor the effect of treatment on a known imbalance that is affecting bodily organ function. The test for electrolytes includes the measurement of sodium, potassium, chloride, and bicarbonate for both diagnosis and management of renal, endocrine, acid-base, water balance, and many other conditions[134]. Potassium used as a most convincing electrolyte marker of renal failure. The combination of decreased filtration and decreased secretion of potassium in distal tubule during renal failure cause increased plasma potassium. Hyperkalemia is the most significant and life-threatening complication of renal failure.

2.13 Hormone of Renal Functions

The kidneys produce three important hormones: erythropoietin, calcitriol (1,25-dihydroxycholecalciferol) and renin. They also synthesize prostaglandins, which affect many processes in the kidneys. In addition to synthesis, the kidneys also contribute to the degradation of certain hormones such as insulin (forms insulinase – cleaves insulin) or parathyroid hormone[135].

a. Erythropoietin:

Erythropoietin is a peptide hormone which regulates erythropoiesis.

Structure and Function

Erythropoietin is a glycoprotein containing 165 amino acids. Its receptors are present on the membranes of red blood cell precursors. Binding of the hormone reduces apoptosis of these cells, multiple cells survive and can therefore complete their development into mature erythrocytes.

Synthesis and Inactivation

In adults, approximately 90 % of erythropoietin is synthesized in the kidneys (interstitial cells), the remaining amount in the liver (perivenous hepatocytes) . The liver plays a key role

in the production of erythropoietin during the fetal period. But in adulthood, the liver is no longer able to compensate for a potential decrease in production in the kidneys.

Clinical Correlations:

For most people in end-stage renal failure, anemia with erythropoietin deficiency occurs. Doctors can administer recombinant erythropoietin to these patients. Erythropoietin is also abused as doping substance – especially in endurance athletics (cycling). The main stimulus for the production of erythropoietin is a decrease in the partial pressure of oxygen in the blood flowing through the two organs. Hormone production is also supported by androgens (testosterone), and catecholamines (β – receptors) [136].

b. Calcitriol (1,25-dihydroxycholecalciferol):

It is a hormone which binds to and activates the vitamin D receptor in the nucleus of the cell, which then increases the expression of many genes. Calcitriol increases blood calcium (Ca^{2+}) mainly by increasing the uptake of calcium from the intestines. Final activation of vitamin D to the active hormone calcitriol takes place in the kidneys – 1-hydroxylation of 25-hydroxycholecalciferol to 1, 25-dihydroxycholecalcifero. Calcitriol stimulates the small intestine for protein synthesis allowing absorption of Ca2+and phosphates. This ensures the availability of Ca^{2+} and phosphate for bone growth. Calcitriol simultaneously activates osteoblasts to synthesize collagen[137].

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It can be given as a medication for the treatment of low blood calcium and hyperparathyroidism due to kidney disease, low blood calcium due to hypoparathyroidisn, osteoporosis, osteomlacia and familial hypophosphatemia, and can be taken by mouth or by injection into a vein. Excessive amounts or intake can result in weakness, headache,

nausea, constipation, urinary tract infections, and abdominal pain.Serious side effects may include high blood calcium and anaphylaxis. Regular blood tests are recommended after the medication is started and when the dose is changed[138].

c. Renin:

Renin is part of the renin – angiotensin – aldosterone system (RAAS). In the case of insufficient blood flow to the kidneys (e.g., decrease in blood volume) cells of the renal juxtaglomerular apparatus begin the synthesis of protein renin. Renin is an enzyme, which catalyzes the conversion of plasmatic angiotensinogen to angiotensin I. Angiotensin I is then converted by angiotensin converting enzyme to angiotensin II, which stimulates aldosterone synthesis and causes vasoconstriction[139].

Renin is a large-ish 37 kDa enzyme which is synthesised by juxtaglomerular cells of the renal cortex. It is produced by cutting chunks out of protein, a slightly larger precursor protein (46kDa). Renin remains in storage vesicles of the juxtaglomerular cells, waiting to be released by a range of stimuli. Its release and serum concentration level is the rate-limiting step in the pathway of RAAS activation, whereas its substrate is present in the blood at relatively high concentrations, around 1.3mg/L[140].

2.14 Effect of SARS-CoV-2 Vaccination on Renal Function

Since the description of the first report of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in December 2019, the upper respiratory tract has been well established to be the main infection site; however, much evidence has demonstrated that other organs, including the heart, liver, and kidneys in addition to the respiratory tract, can be severely affected. Acute kidney injury (AKI) caused by the new coronavirus is associated with the severe clinical status of patients and, consequently, a worse prognosis. Although the

mechanism of injury is not completely understood, it is currently known to go beyond acute tubular necrosis secondary to hemodynamic instability in critically ill patients[141].

Patients with chronic kidney disease (CKD) are at high risk for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections. Newly developed SARS-CoV-2 vaccines have been proven to be effective against infection during the Covid-19 pandemic. The vaccines are safe for patients with CKD, with only sporadic cases of nephrotic syndrome or acute kidney injury (AKI) having been reported following Covid-19 vaccination[142]. Vaccination against Covid-19 reduces mortality and risks of hospitalization from Covid-19 in the general population. KTRs on immunosuppression have been demonstrated to have attenuated serologic response to conventional two-dose Covid-19 vaccination schedule. Serologic response to vaccination in patients treated with HD is variable, with most studies suggesting that vaccination induces anti-Spike antibodies. Many countries recognized patients undergoing HD and KTRs as "clinically extremely vulnerable" and prioritized early vaccination of patients requiring KRT[143].

The binding of the viral S protein to the angiotensin-converting enzyme 2 (ACE2) receptor on the surface of host cells triggers SARS-CoV-2 infection. These receptors are in large quantities in type II pneumocytes in the lungs, heart, and kidneys. The virus incorporation into the cell occurs when proteins present on the surface of the virus, called "spikes," bind to ACE2 and are endocytosed by activating transmembrane serine protease type 2 (TMPRSS 2), which starts the intracellular viral replication[144].

The infection course, morbidity, and mortality have changed favorably since the advent of vaccines against the new coronavirus in the last year, drastically reducing the number of deaths. However, with the spread of vaccination, adverse effects of vaccines, including kidney injury, have generated concerns globally. Since the beginning of large-scale

immunization, the publication of case series of renal diseases with the emergence of new glomerulopathies or reactivation of previous glomerulopathies has increased[145].

2.15 Mechanisms of Renal Entry and Kidney Injury in COVID-19 Infection

SARS-CoV-2 mainly binds ACE2 proteins, which are expressed in kidneys on the brush border of the apical membrane of proximal tubules and to a lesser extent in podocytes[146]. Thus, it could be hypothesized that the virus enters the arteriole and the glomerular capillaries and initially infects the glomerular endothelial cells. Consequently, podocytes are infected, and the virus enters the tubular fluid and binds to its receptors in proximal tubules, leading to acute tubular necrosis protein leakage in Bowman's capsule, collapsing glomerulopathy, and mitochondrial impairment[147]. Initially, the virus gains access to the kidneys through the bloodstream, whereby many COVID-19 patients were reported to have SARS-CoV-2 RNAemia. Although viremia in COVID-19 subjects remains a matter of debate, the virus was found in extracellular vesicles, allowing a systemic spread across the body and damage of various organs, particularly the kidneys. Of concern is the ability of the SARS-CoV-2 to integrate its RNA within the genomic DNA of the host cells after reverse transcription[148]. This could possibly translate into the expression of viral proteins in kidney cells, a transformation that could lead to autoimmune disease. Thus, the first step of SARS-CoV-2 infection in humans is the contact of the virus with cell-surface ACE2. ACE2 interacts with external SARS-CoV-2 by binding to the receptor-binding domain (RBD) of the viral spike protein. This process is followed by proteolytic cleavage of the spike protein, which allows fusion to cells, and transmembrane protease serine 2 (TMPRSS2) has been identified as a protease responsible for the reaction[149].

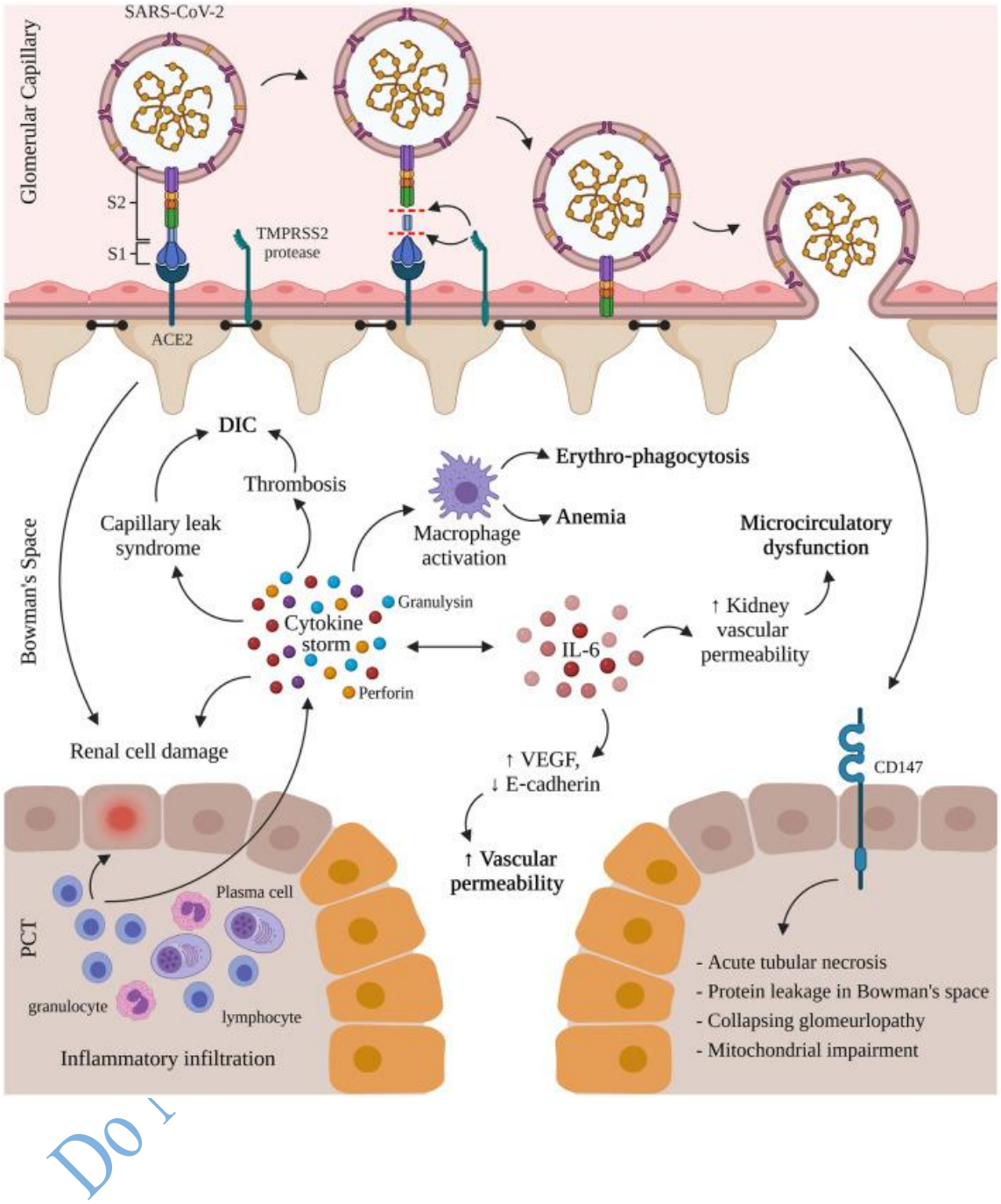

Figure 2.7: Mechanisms of renal entry and kidney injury in COVID-19 infection

Source: Reviews in medical virology, 2021[145].

COVID-19 infection in humans proceeds by the interaction of the receptor-binding domain(RBD) of the viral spike protein with the cell-surface angiotensin-converting enzyme II (ACE2). This is followed by the proteolytic cleavage of the spike protein through proteaseslike the transmembrane protease serine 2 (TMPRSS2)[150]. The virus interacts with CD147, expressed on the proximal convoluted tubules (PCT) of the nephron and on infiltratinginflammatory cells, resulting in acute tubular necrosis, protein leakage in Bowman's capsule, collapsing glomerulopathy, and mitochondrial impairment. Simultaneously, the activated lymphocytes from the inflammatory infiltrates (lymphocytes, plasma cells and eosinophils) in the renal interstitium destroy renal cells and induce a cytokine storm of perforin, granulysin, and proinflammatory cytokines[151].

The cytokine storm activates macrophages leading to erythro-phagocytosis and anemia, induces capillary leak syndrome and thrombosis both linked to disseminated intravascular coagulation (DIC), and contributes to renal cell damage also caused by direct renal infection[152]. Oversecretion of key cytokine, interleukin-6 (IL-6), that binds the IL-6 receptor and activates the vascular endothelial growth factor (VEGF), decreases the expression of E-cadherin, increases vascular permeability, shock, and MOD while increasing kidney vascular permeability and microcirculatory dysfunction[153].

2.16 Thyroid Gland

The thyroid gland is a small gland with significant effects. The mature thyroid gland is located in the neck and is responsible for delivering hormones to the body[154]. Hormones released from the thyroid include thyroxine and calcitonin, which have an impact on the body's basal metabolic rate, heart, brain, muscle, digestive tract, and calcium homeostasis. The thyroid is the body's first endocrine gland to develop, with development beginning around the third week of gestation.

The thyroid arises from the pharyngeal pouches, which are composed of endoderm. Thyroid development begins as a diverticular outgrowth from the primitive pharynx. The diverticulum then descends inferiorly to reach its final destination in the neck. Traditionally, the thyroid is located inferior to the thyroid cartilage, approximately at the level of the C5-T1 vertebrae. During its descent, the thyroid connects to the tongue by the thyroglossal duct[155].

The thyroid originates between the first and second pharyngeal pouches near the base of the tongue. In the third week of gestation, around day 20-24, endodermal cells of the primitive pharynx proliferate, creating the thyroid diverticulum. Beginning in the fifth week of gestation, the thyroid diverticulum migrates caudally along the midline, crossing anteriorly to the hyoid bone and laryngeal cartilage[156]. During migration, the thyroid remains attached to the tongue via the thyroglossal duct. In early descent, the thyroid is hollow but then solidifies during migration forming the follicular elements of the thyroid. Division of the thyroid into right and left lobes occurs in the fifth week of gestation.

Also, during the fifth week, the ultimobranchial bodies arise from the fourth/fifth pharyngeal pouches. The ultimobranchial bodies ultimately differentiate into the parafollicular C-cells, which play an essential role in calcium homeostasis. Traditionally, the parafollicular C-cells were thought to arise from neural crest cells, but recent works have disputed this by suggesting they arise from endoderm. The ultimobranchial bodies fuse with the superior dorsolateral aspect of the developing thyroid, forming Zuckerkandl's tubercle[157]. The parafollicular C-cells then disseminate into the thyroid, but generally remain limited to the superolateral aspects of the thyroid, while the lower-third of the thyroid remains mostly devoid of C-cells. Fully developed C-cells secrete calcitonin, which decreases serum calcium by inhibiting osteoclast function.

By the seventh week of gestation, the thyroid has reached its final destination in the neck. Normally, the thyroglossal duct degenerates by the tenth week of gestation with only the foramen cecum to indicate its former existence. In some instances, incomplete obliteration of the duct can lead to abnormalities, including thyroglossal duct cysts, lingual thyroid, or a pyramidal lobe. Cellular differentiation and maturation then continue until the thyroid is functionally mature by the twelfth gestational week[158].

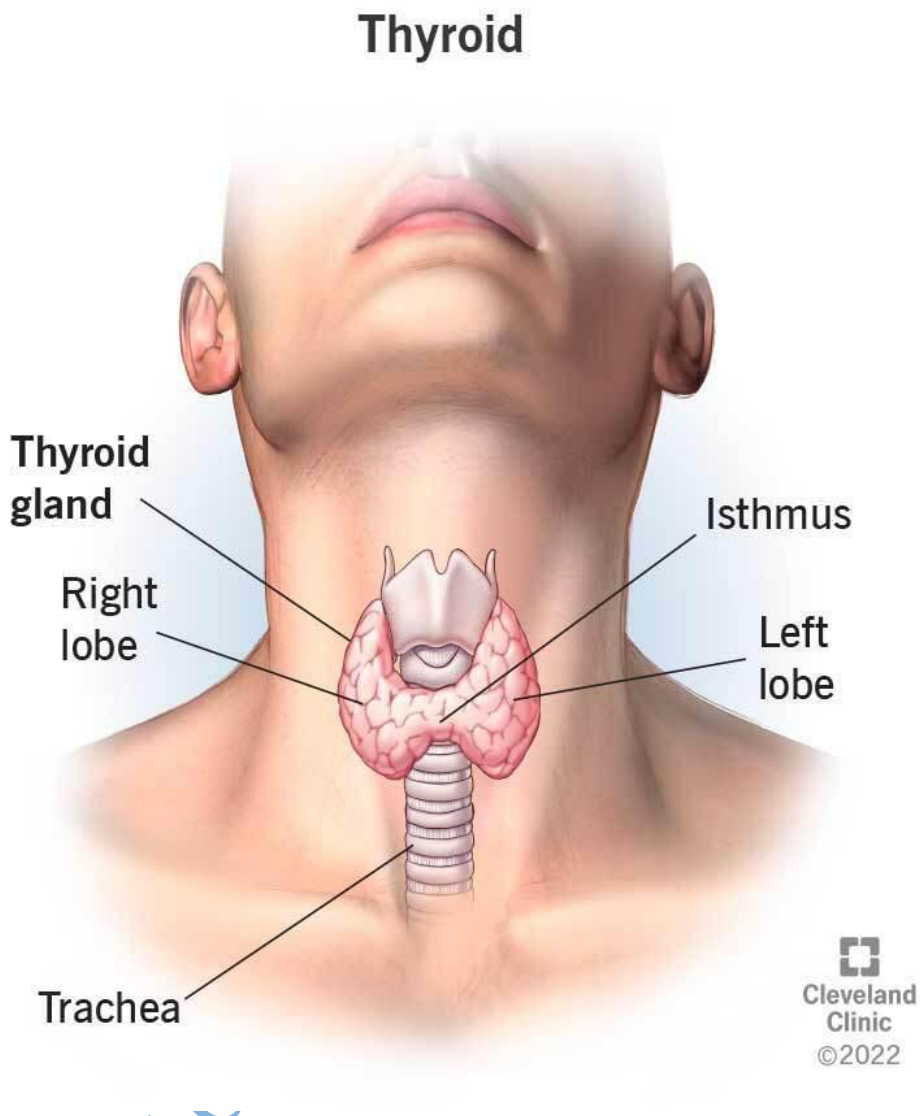

Figure 2.8: The thyroid gland

Source: Anatomy, 2022[161]

2.16.1 Gross Anatomy of Thyroid Gland

The thyroid gland is divided into two lobes that are connected by the isthmus, which crosses the midline of the upper trachea at the second and third tracheal rings. In its anatomic position, the thyroid gland lies posterior to the sternothyroid and sternohyoid muscles, wrapping around the cricoid cartilage and tracheal rings. It is located inferior to the laryngeal thyroid cartilage, typically corresponding to the vertebral levels C5-T1. The thyroid attaches to the trachea via a consolidation of connective tissue, referred to as the lateral suspensory ligament or Berry's ligament. This ligament connects each of the thyroid lobes to the trachea. The thyroid gland, along with the esophagus, pharynx, and trachea, is found within the visceral compartment of the neck which is bound by pretracheal[159].

The "normal" thyroid gland has lateral lobes that are symmetrical with a well-marked centrally located isthmus. The thyroid gland typically contains a pyramidal extension on the posterior-most aspect of each lobe, referred to as the tubercle of Zuckerkandl. Despite these general characteristics, the thyroid gland is known to have many morphologic variations. The position of the thyroid gland and its close relationship with various structures brings about several surgical considerations with clinical relevance[160].

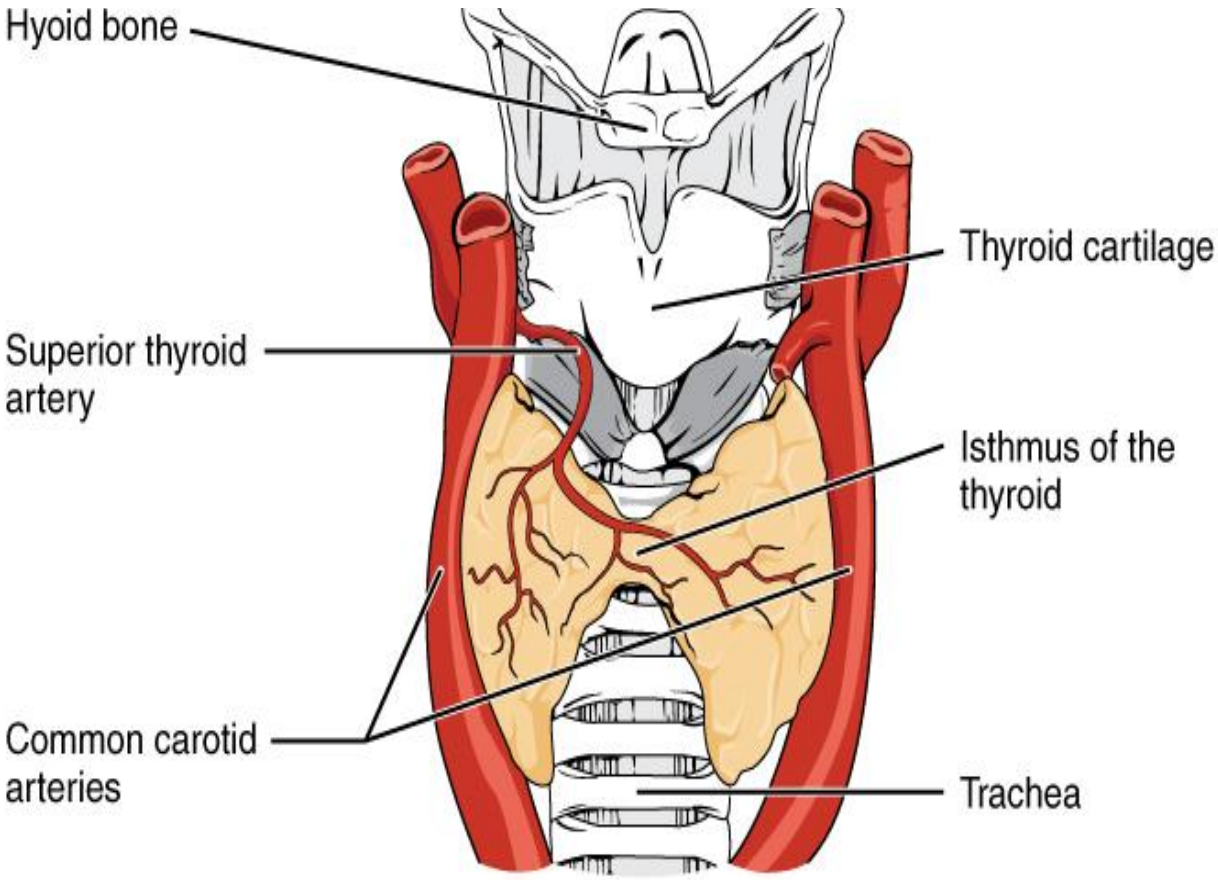

Figure 2.9: The thyroid gland

Source: Anatomy, 2022[161]

The thyroid gland is a butterfly-shaped organ composed of two lobes, left and right, connected by a narrow tissue band, called an "isthmus". It weighs 25 grams in adults, with each lobe being about 5 cm long, 3 cm wide, and 2 cm thick and the isthmus about 1.25 cm in height and width. The gland is usually larger in women than in men, and increases in size during pregnancy.

The thyroid is near the front of the neck, lying against and around the front of the larynx and trachea[161]. The thyroid cartilage and cricoid cartilage lie just above the gland, below the Adam's apple. The isthmus extends from the second to third rings of the trachea, with the uppermost part of the lobes extending to the thyroid cartilage and the lowermost around the fourth to sixth tracheal rings. The infrahyoid muscles lie in front of the gland and the sternocleidomastoid muscle to the side. Behind the outer wings of the thyroid lie the two carotid arteries. The trachea, larynx, lower pharynx and esophagus all lie behind the thyroid. In this region, the recurrent laryngeal nerve and the inferior thyroid artery pass next to or in the ligament. Typically, four parathyroid glands, two on each side, lie on each side between the two layers of the thyroid capsule, at the back of the thyroid lobes[162].

The thyroid gland is covered by a thin fibrous capsule, which has an inner and an outer layer. The inner layer extrudes into the gland and forms the septa that divide the thyroid tissue into microscopic lobules. The outer layer is continuous with the pretracheal fascia, attaching the gland to the cricoid and thyroid cartilages via a thickening of the fascia to form the posterior suspensory ligament of thyroid gland, also known as Berry's ligament[163].

2.16.1.1 Blood, lymph and nerve supply of the thyroid gland

The thyroid gland has an extremely rich blood supply and is estimated to be six times as vascular as the kidney and relatively three to four times more vascular than the brain. It receives blood from the superior and inferior thyroid arteries[164]. These paired vessels supply the superior and inferior aspect of the gland. The superior thyroid artery is the first branch of

the external carotid artery as it arises near the level of the superior horn of the thyroid cartilage. The superior thyroid artery then moves anterior, inferior, and towards the midline behind the sternothyroid muscle to the superior pole of the lobe of the thyroid gland. From this point, the superior thyroid artery branches off[165]. One branching point runs down the dorsal aspect of the thyroid gland. The other superficial branch runs along the sternothyroid muscle and thyrohyoid muscles, supplying branches to these muscles as well as the sternohyoid. The superficial branch continues downward to further give off the cricothyroid branch and to supply the isthmus, inner sides of the lateral lobes, and when present the pyramidal lobe[166].

The thyrocervical trunk arises from the anterosuperior surface of the subclavian artery and gives rise to three branches, one being the inferior thyroid artery. The inferior thyroid artery branches from the thyrocervical trunk at the inner border of the anterior scalene muscle and advances medially to the thyroid gland. The artery reaches the posterior surface of the lateral lobe of the thyroid gland at the level of the junction of the upper two thirds and lower third of the outer border. The largest branch of the inferior thyroid artery is the ascending cervical branch, and it is important not to mistake this branch for the inferior thyroid artery itself[167].

In 10% of the population, there is an additional artery known as the thyroid ima artery. This artery has a variable origin including the brachiocephalic trunk, aortic arch, the right common carotid, the subclavian, the pericardiacophrenic artery, the thyrocervical trunk, transverse scapular, or internal thoracic artery[168]. The thyroid ima most commonly originates from the brachiocephalic trunk and supplies the isthmus and anterior thyroid gland. The thyroid gland is drained via the superior, middle, and inferior thyroid veins. The middle and superior thyroid veins follow a tortuous route and eventually drain into the internal jugular vein on either side of the neck. The drainage of the inferior thyroid vein may enter either the subclavian or brachiocephalic veins, located just posterior to the manubrium. Lymphatic

drainage of the thyroid gland involves the lower deep cervical, prelaryngeal, pretracheal, and paratracheal nodes. The paratracheal and lower deep cervical nodes, specifically, receive lymphatic drainage from the isthmus and the inferior lateral lobes. The superior portions of the thyroid gland drain into the superior pretracheal and cervical nodes[169].

2.16.2 Microscopic Anatomy of Thyroid Gland

Microscopic spherical sacs called thyroid follicles make up most of the thyroid gland. The wall of each follicle consists primarily of cells called follicular cells, most of which extend to the lumen (internal space) of the follicle[170]. A basement membrane surrounds each follicle. When the follicular cells are inactive, their shape is low cuboidal to squamous, but under the influence of TSH they become active in secretion and range from cuboidal to low columnar in shape. The follicular cells produce two hormones: thyroxine, which is also called tetraiodothyronine or T4 because it contains four atoms of iodine and triiodothyronine or T3, which contains three atoms of iodine. T3 and T4 together are also known as thyroid hormones[171]. A few cells called parafollicular cells or C cells lie between follicles, they produce the hormone calcitonin, which helps regulate calcium homeostasis. The center, or lumen, of each thyroid follicle is filled with proteins called thyroglobulin synthesized and secreted by cells of the thyroid follicles. Large amounts of the thyroid hormones are stored in the thyroid follicles as part of the thyroglobulin molecules. Between the follicles, a delicate network of loose connective tissue contains numerous capillaries. Scattered parafollicular cells are found between the follicles and among the cells that make up the walls of the follicle[172].

2.17 Physiology of the Thyroid Gland

The thyroid secretes two major hormones, thyroxine and triiodothyronine, commonly called T4 and T3, respectively. Both of these hormones profoundly increase the metabolic rate of the body[173]. Complete lack of thyroid secretion usually causes the basal metabolic rate to fall

40 to 50 percent below normal, and extreme excesses of thyroid secretion can increase the basal metabolic rate to 60 to 100 percent above normal. Thyroid secretion is controlled primarily by thyroid-stimulating hormone (TSH) secreted by the anterior pituitary gland[174]. The thyroid gland also secretes calcitonin, an important hormone for calcium metabolism. About 93 percent of the metabolically active hormones secreted by the thyroid gland are thyroxine, and 7 percent triiodothyronine. However, almost all the thyroxine is eventually converted to triiodothyronine in the tissues, so both are functionally important. The functions of these two hormones are qualitatively the same, but they differ in rapidity and intensity of action. Triiodothyronine is about four times as potent as thyroxin, but it is present in the blood in much smaller quantities and persists for a much shorter time than thyroxine[175].

The thyroid gland is composed of closed follicles (100 to 300 micrometers in diameter) filled with a secretory substance called colloid and lined with cuboidal epithelial cells that secrete into the interior of the follicles[173]. The major constituent of colloid is the large glycoprotein thyroglobulin, which contains the thyroid hormones. Once the secretion has entered the follicles, it must be absorbed back through the follicular epithelium into the blood before it can function in the body. The thyroid gland has a blood flow about five times the weight of the gland each minute, which is a blood supply as great as that of any other area of the body, with the possible exception of the adrenal cortex. To form normal quantities of thyroine, about 50milligrams of ingested iodine in the form of iodides are required each year, or about 1 mg/week. To prevent iodine deficiency, common table salt is iodized with about 1 part sodium iodide to every 100,000 parts sodium chloride. Iodides ingested orally are absorbed from the gastrointestinal tract into the blood in about the same manner as chlorides[177].

Normally, most of the iodides are rapidly excreted by the kidneys, but only after about fifth are selectively removed from the circulating blood by the cells of the thyroid gland and used for synthesis of the thyroid hormones. The process of concentrating the iodide in the cell is

called iodide trapping[178]. In a normal gland, the iodide pump concentrates the iodide to about 30 times its concentration in the blood. When the thyroid gland becomes maximally active, this concentration ratio can rise to as high as 250 times The rate of iodide trapping by the thyroid is influenced by several factors, the most important being the concentration of TSH from the anterior pituitary gland being stimulated by Thyroid Releasing Hormone (TRH) from the Hypothalamus[179].

Thyroid hormones have long half-life T4 has a long half-life of 7 days. Half-life ofT3 is varying between 10 and 24 hours.

Rate of Secretion:

Thyroxine- 80 to 90 ug/day

Tri-iodothyronine = 4 to 5 ug/day

Reverse T3 =1 to 2 ug/day.

Plasma Level:

Total T3 =0.12 ug/dL

Total T4 =8 ug/dL.

The hormones of the thyroid gland are stored in the form of vesicles within thyroglobulin after synthesis and are stored for a long period. Each thyroglobulin molecule contains 5 or 6 molecules of thyroxine. There is also an average of l tri-iodothyronine molecule for every 10 molecules of thyroxine. In combination with thyroglobulin, the thyroid hormones can be stored for several months[179]. Thyroid gland is unique in this, as it is the only endocrine gland that can store its hormones for a long period of about 4 months. So, when the synthesis of thyroid hormone stops, the signs and symptoms of deficiency do not appear for about 4 months. Thyroid hormones are transported in the blood by three types of proteins:

1. Thyroxine-binding globulin (TBG)

2. Thyroxine-binding prealbumin (TBPA)

3. Albumin.

Thyroid hormones have two major effects on the body:

I. To increase basal metabolic rate

II. To stimulate growth in children.

Other actions of thyroid hormones include:

- Thyroxine increases the metabolic activities in most of the body tissues, except brain, retina, spleen, testes and lungs. It increases Basal Metabolic Rate (BMR) by increasing the oxygen consumption of the tissues. The action that increases the BMR is called calorigenic action. In hyperthyroidism, BMR increases by about 60% to 100% above the normal level and in hypothyroidism it falls by 20% to 40% below the normal level[179].

- Thyroid hormone increases the synthesis of proteins, stimulate almost all the processes involved in the metabolism of carbohydrates and also fat metabolism into in the cells.

- Even though there is an increase in the blood level of free fatty acids, thyroxine specifically decreases the cholesterol, phospholipids and triglyceride levels in plasma. So, in hyposecretion of thyroxine, the cholesterol level in plasma increases, resulting in atherosclerosis. Thyroxine also increases deposition of fats in the liver, leading to fatty liver. Thyroxine decreases plasma cholesterol level by increasing its excretion from liver cells into bile. Cholesterol enters the intestine through bile and then it is excreted through the feces.

- Thyroid hormone increases the heat production in the body, by accelerating various cellular metabolic processes. During hypersecretion of thyroxine, the body temperature increases greatly, resulting in excess sweating. Normal thyroxine level is necessary to maintain normal sleep pattern. Hypersecretion of thyroxine causes excessive stimulation of the muscles and central nervous system. So, the person feels tired, exhausted and feels like sleeping. But, the person cannot sleep because of the stimulatory effect of thyroxine on neurons. On the other hand, hyposecretion of thyroxine causes somnolence.

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- Thyroxine is essential for the normal activity of skeletal muscles. Slight increase in thyroxine level makes the muscles to work with more vigor. But, hypersecretion of thyroxine causes weakness of the muscles due to catabolism of proteins. This condition is called thyrotoxicmyopathy. The muscles relax very slowly after the contraction. Hyperthyroidism also causes fine muscular tremor. Tremor occurs at the frequency of 10 to 15 times per second. It is due to the thyroxine-induced excess neuronal activity, which controls the muscle. The lack of thyroxine makes the muscles more sluggish.

- Normal thyroxine level is essential for normal sexual function. In men, hypothyroidism leads to complete loss of libido (sexual drive) and hyperthyroidism leads to impotence. In women, hypothyroidism causes menorrhagia and polymenorrhea. In some women, it causes irregular menstruation and occasionally amenorrhea. Hyperthyroidism in women leads to oligomenorrhea and sometimes amenorrhea[180].

- Generally, thyroxine increases the appetite and food intake. It also increases the secretions and movements of GI tract. So, hypersecretion of thyroxine causes diarrhea and the lack of thyroxine causes constipation.

- Thyroxine increases the rate and force of respiration indirectly. The increased metabolic rate (caused by thyroxine) increases the demand for oxygen and formation of excess

carbon dioxide, These two factors stimulate the respiratory centers to increase the rate and force of respiration.

- Thyroxine increases the demand for secretion by other endocrine glands.

Diseases of the thyroid gland include:

1. Hyperthyroidism is usually caused by Toxic Goiter or Thyrotoxicosis or Grave's disease, autoimmunity and thyroid adenoma. The symptoms of hyperthyroidism are:

a) A high state of excitability,

b) Intolerance to heat,

c) Increased sweating,

d) Mild to extreme weight loss (sometimes as much as 100 pounds),

e) Varying degrees of diarrhea,

f) Muscle weakness,

g) Nervousness or other psychic disorders,

h) Extreme fatigue

h) but inability to sleep,

i) Tremor of the hands.

j) Exophthalmos. Most people with hyperthyroidism develop some degree of protrusion of the eyeballs,

2. Hypothyroidisn, like hyperthyroidism, is often initiated by autoimmunity against the droid gland (Hashimoto disease), but immunity that destroys the gland rather than stimulates it. The thyroid glands of most of these patients first have autoimmune thyroiditis," which means

thyroid inflammation[178]. This causes progressive deterioration and finally fibrosis of the gland, with resultant diminished or absent secretion of thyroid hormone. Several other types of hypothyroidism also occur, often associated with development of enlarged thyroid glands, called thyroid goiter which could be Endemic Colloid Goiter Caused by Dietary Iodide Deficiency or Idiopathic Nontoxic Colloid Goiter. Enlarged thyroid glands similar to those of endemic colloid goiter can also occur in people who do not have iodine deficiency. Physiologic characteristics of hypothyroidism include:

a) Fatigue and extreme somnolence with sleeping up to 12 to 14 hours a day, extreme muscular sluggishness, slowed heart rate, decreased cardiac output, decreased blood volume, sometimes increased body weight, constipation, mental sluggishness, failure of many trophic functions in the body evidenced by depressed growth of hair and scaliness of the skin, development of a froglike husky voice, and, in severe cases, development of an edematous appearance throughout the body called myxedema[182].

b) Cretinism

c) Atherosclerosis in hypothyroidism

2.18 Pathophysiology of Thyroid Gland

The specific role of thyroid-stimulating hormone in its pathogenesis has not been unraveled. It has been variously defined and characterized by the increased volume of the thyroid gland with the formation of multiple nodules. Although a number of definitions exist, the most accepted is the thyroid gland weighing over 20–25 g or a volume of over 19 ml in females and 25 ml in males[183].

a. Pyramidal Lobe of the Thyroid (PL)

Pyramidal lobes occur when the distal portion of the thyroglossal duct differentiates into thyroid tissue. Estimates of pyramidal lobe incidence vary. For example, a 2015 study of 166

thyroidectomy patients found a pyramidal lobe in 65.7% of patients, with equal gender distribution, while a 2010 study of 90 male cadavers found a pyramidal lobe in 37.8% of patients. Pyramidal lobes most commonly arise from the isthmus but can originate from either lobe (more often the left). They achieve variable lengths but are categorized as short (less than 15 mm), medium (15 to 30 mm) or long (greater than 30 mm), and are commonly longer in females than males. It is considered a normal anatomic variant and is generally asymptomatic[184].

b. Double Pyramidal Lobe (DPL)

An extremely rare variant with only a handful of cases reported. These present with two pyramidal lobes arising from the thyroid, often located at the junction of the isthmus and the right and left lobes, respectively. All case reports involve female patients aged 45 to 58 years old[185].

c. Sub-Isthmic Accessory Gland

Another rare variant of thyroid development. Unlike pyramidal lobes which present superiorly to the thyroid gland, relating to the thyroid descent along the thyroglossal duct, these present inferiorly to the thyroid[186].

d. Absent Isthmus:

A normal thyroid isthmus is commonly located at the second and third tracheal rings, but can vary from the cricoid cartilage down to the fourth tracheal ring. In rare cases, the isthmus is absent. While the right and left lobes are separated, they are often functional[187].

e. Ectopic Thyroid Tissue

Thyroid tissue located outside the normal anatomic position in the anterior neck. It most commonly occurs secondary to failure of embryologic descent. The most common location

for ectopic thyroid tissue is the base of the tongue (at the foramen cecum, secondary to failure or migration), but is also commonly found along the thyroglossal duct, resulting in a high cervical thyroid (due to incomplete migration)[188]. Excessive migration leads to superior mediastinal or pericardiac thyroid tissue. Less commonly, ectopic thyroid tissue can be found in the adrenal/pituitary glands along with the GI system, within the female reproductive system and even within the iris of the eye. One or more foci of ectopic tissue may be present. Ectopic thyroid tissue can be distinguished from thyroglossal duct cysts via thyroid scintigraphy, as ectopic tissue is functional, and cysts contain insufficient thyroid tissue for an examiner to identify[189].

f. Lingual Thyroid:

Rarely, the thyroid fails to descend properly down the thyroglossal duct to the final anatomic position, which leads to retention of thyroid tissue at the foramen cecum, at the base of the tongue, and is more common in female patients. Lingual thyroid can span a wide range of clinical presentations, from an incidental finding to partial airway obstruction. If symptomatic, lingual thyroid tissue can be surgically removed - with a risk of hypothyroidism if insufficient thyroid tissue remains[190].

g. Thyroglossal Duct Cyst

Relatively rare, present in approximately 7% of the population worldwide. Arises when a portion of the thyroglossal duct fails to involute. Secretions from the epithelial lining result in inflammation and cyst formation[191]. Thyroglossal cysts are found in the midline and closely associated with the hyoid bone, with 20 to 25% found suprahyoid and 25-65% infrahyoid. Cysts are frequently asymptomatic but can become infected and present as an abscess. Classically, thyroglossal duct cysts will elevate with swallowing or protrusion of the tongue. Thyroglossal cysts are often confused with branchial cleft cysts, which arise from

incomplete involution of pharyngeal clefts. The second cleft is the one most commonly implicated, but third and fourth pouches may also occur. Branchial cleft cysts typically present lateral to the midline, often anterior to the sternocleidomastoid. They do not elevate with swallowing or tongue protrusion[192].

h. Lingual Cyst

This is a thyroglossal duct cyst occurring at the base of the tongue. These can present with difficulty in swallowing or breathing[193].

2.19 Hormone of Thyroid Gland

The thyroid hormone is well known for controlling metabolism, growth, and many other bodily functions. The thyroid gland, anterior pituitary gland, and hypothalamus comprise a self-regulatory circuit called the hypothalamic-pituitary-thyroid axis[194]. The main hormones produced by the thyroid gland are thyroxine or tetraiodothyronine (T4) and triiodothyronine (T3). Thyrotropin-releasing hormone (TRH) from the hypothalamus, thyroid-stimulating hormone (TSH) from the anterior pituitary gland, and T4 work in synchronous harmony to maintain proper feedback mechanism and homeostasis. Hypothyroidism, caused by an underactive thyroid gland, typically manifests as bradycardia, cold intolerance, constipation, fatigue, and weight gain. In contrast, hyperthyroidism caused by increased thyroid gland function manifests as weight loss, heat intolerance, diarrhea, fine tremor, and muscle weakness[195].

Iodine is an essential trace element absorbed in the small intestine. It is an integral part of T3 and T4. Sources of iodine include iodized table salt, seafood, seaweed, and vegetables. Decreased iodine intake can cause iodine deficiency and decreased thyroid hormone synthesis. Iodine deficiency can cause cretinism, goiter, myxedema coma, and hypothyroidism[196].

a. Cellular Level

Regulation of thyroid hormone starts at the hypothalamus. The hypothalamus releases thyrotropin-releasing hormone (TRH) into the hypothalamic-hypophyseal portal system to the anterior pituitary gland. TRH stimulates thyrotropin cells in the anterior pituitary to the release of thyroid-stimulating hormone (TSH). TRH is a peptide hormone created by the cell bodies in the periventricular nucleus (PVN) of the hypothalamus. These cell bodies project their neurosecretory neurons down to the hypophyseal portal circulation, where TRH can concentrate before reaching the anterior pituitary[197].

TRH is a tropic hormone, meaning that it indirectly affects cells by stimulating other endocrine glands first. It binds to the TRH receptors on the anterior pituitary gland, causing a signal cascade mediated by a G-protein coupled receptor[198]. Activation of Gq protein leads to the activation of phosphoinositide-specific phospholipase C (PLC). PLC hydrolyzes phosphatidylinositol 4,5-P (PIP) into inositol 1,4,5-triphosphate (IP) and 1,2-diacylglycerol (DAG). These second messengers mobilize intracellular calcium stores and activate protein kinase C, leading to downstream gene activation and transcription of TSH. TRH also has a non-tropic effect on the pituitary gland through the hypothalamic-pituitary-prolactin axis. As a non-tropic hormone, TRH directly stimulates lactotropic cells in the anterior pituitary to produce prolactin. Other substances like serotonin, gonadotropin-releasing hormone, and estrogen can also stimulate prolactin release. Prolactin can cause breast tissue growth and lactation[199].

TSH is released into the blood and binds to the thyroid-releasing hormone receptor (TSH-R) on the basolateral aspect of the thyroid follicular cell. The TSH-R is a Gs-protein coupled

receptor, and its activation leads to the activation of adenylyl cyclase and intracellular levels of cAMP. The increased cAMP activates protein kinase A (PKA). PKA phosphorylates different proteins to modify their functions. The five steps of thyroid synthesis are below:

1. **Synthesis of Thyroglobulin**: Thyrocytes in the thyroid follicles produce a protein called thyroglobulin (TG). TG does not contain any iodine, and it is a precursor protein stored in the lumen of follicles. It is produced in the rough endoplasmic reticulum. Golgi apparatus pack it into the vesicles, and then it enters the follicular lumen through exocytosis.

2. **Iodide uptake**: Protein kinase A phosphorylation causes increased activity of basolateral Na+-I- symporters, driven by Na+-K+-ATPase, to bring iodide from the circulation into the thyrocytes. Iodide then diffuses from the basolateral side to the apex of the cell, where it is transported into the colloid through the pendrin transporter[199].

3. **Iodination of thyroglobulin**: Protein kinase A also phosphorylates and activates the enzyme thyroid peroxidase (TPO). TPO has three functions: oxidation, organification, and coupling reaction.

1. **Oxidation:** TPO uses hydrogen peroxide to oxidize iodide (I-) to iodine (I2). NADPH-oxidase, an apical enzyme, generates hydrogen peroxide for TPO.

2. **Organification:** TPO links tyrosine residues of thyroglobulin protein with I2. It generates monoiodotyrosine (MIT) and diiodotyrosine (DIT). MIT has a single tyrosine residue with iodine, and DIT has two tyrosine residues with iodine.

3. **Coupling Reaction:** TPO combines iodinated tyrosine residues to make triiodothyronine (T3) and tetraiodothyronine (T4). MIT and DIT join to form T3, and two DIT molecules form T4.

4. **Storage**: thyroid hormones are bound to thyroglobulin for stored in the follicular lumen.

5. **Release**: thyroid hormones are released into the fenestrated capillary network by thyrocytes in the following steps:

1. Thyrocytes uptake iodinated thyroglobulin via endocytosis
2. Lysosome fuse with the endosome containing iodinated thyroglobulin
3. Proteolytic enzymes in the endolysosome cleave thyroglobulin into MIT, DIT, T3, and T4.
4. T3 (20%) and T4 (80%) are released into the fenestrated capillaries via MCT8 transporter.
5. Deiodinase enzymes remove iodine molecules from DIT and MIT. Iodine can be salvaged and redistributed to an intracellular iodide pool

b. Organ Systems Involved

Thyroid hormone affects virtually every organ system in the body, including the heart, CNS, autonomic nervous system, bone, GI, and metabolism. In general, when the thyroid hormone binds to its intranuclear receptor, it activates the genes for increasing metabolic rate and thermogenesis. Increasing metabolic rate involves increased oxygen and energy consumption[200].

Heart: thyroid hormones have a permissive effect on catecholamines. It increases the expression of beta-receptors to increase heart rate, stroke volume, cardiac output, and contractility.

Lungs: thyroid hormones stimulate the respiratory centers and lead to increased oxygenation because of increased perfusion.

Skeletal muscles: thyroid hormones cause increased development of type II muscle fibers. These are fast-twitch muscle fibers capable of fast and powerful contractions.

Metabolism: thyroid hormone increases the basal metabolic rate. It increases the gene expression of Na+/K+ ATPase in different tissues leading to increased oxygen consumption, respiration rate, and body temperature. Depending on the metabolic status, it can induce lipolysis or lipid synthesis. Thyroid hormones stimulate the metabolism of carbohydrates and anabolism of proteins. Thyroid hormones can also induce catabolism of proteins in high doses. Thyroid hormones do not change the blood glucose level, but they can cause increased glucose reabsorption, gluconeogenesis, glycogen synthesis, and glucose oxidation[201].

Growth during childhood: In children, thyroid hormones act synergistically with growth hormone to stimulate bone growth. It induces chondrocytes, osteoblasts, and osteoclasts. Thyroid hormone also helps with brain maturation by axonal growth and the formation of the myelin sheath.

Function

- Increases the basal metabolic rate
- Depending on the metabolic status, it can induce lipolysis or lipid synthesis.
- Stimulate the metabolism of carbohydrates
- Anabolism of proteins. Thyroid hormones can also induce catabolism of proteins in high doses.
- Permissive effect on catecholamines
- In children, thyroid hormones act synergistically with growth hormone to stimulate bone growth.
- The impact of thyroid hormone in CNS is important. During the prenatal period, it is needed for the maturation of the brain. In adults, it can affect mood. Hyperthyroidism can

lead to hyperexcitability and irritability. Hypothyroidism can cause impaired memory, slowed speech, and sleepiness.

- Thyroid hormone affects fertility, ovulation, and menstruation.

2.20 Mechanism of Action of Thyroid Hormone

Thyroid hormones are lipophilic and circulate bound to the transport proteins. Only a fraction (approximately 0.2%) of the thyroid hormone (free T4) is unbound and active. Transporter proteins include thyroxine-binding globulin (TBG), transthyretin, and albumin[202]. TBG transports the majority (two-thirds) of the T4, and transthyretin transports thyroxine and retinol. When it reaches its target site, T3 and T4 can dissociate from their binding protein to enter cells either by diffusion or carrier-mediated transport. Receptors for T3 bind are already bound to the DNA in the nucleus before the ligand binding. T3 or T4 then bind to nuclear alpha or beta receptors in the respective tissue and cause activation of transcription factors leading to the activation of certain genes and cell-specific responses. Thyroid hormones are degraded in the liver via sulfation and glucuronidation and excreted in the bile[203].

Thyroid receptors are transcription factors that can bind to both T3 and T4. However, they have a much higher affinity for T3. As a result, T4 is relatively inactive. Deiodinases convert T4 to active T3 or inactive reverse T3 (rT3). There are three types of deiodinases: type I, II, and III. Type I (DIO1) and II (DIO2) are located in the liver, kidneys, muscles, and thyroid glands. Type III (DIO3) deiodinases are located in the CNS and placenta. DIO1 and DIO2 convert T4 to the active form T3, and DIO3 converts T4 into inactive form rT3[204].

2.21 Mechanism of the Effect of SARS-CoV-2 Infection on Thyroid Function

SARS-CoV-2 enters the lungs and into the lung parenchyma through the respiratory system. Eventually, spike proteins of the virus attach to angiotensin-converting enzyme 2 (ACE2), which is expressed at the surface of pneumocytes[205]. ACE2 binds to the spike proteins of SARS-CoV-2 and acts as a receptor, mediating the entry of the virus to the host cells. This virus mechanism is also used to gain entry to other cell types of the human body. Many endocrine system organs have ACE2-expressing cells, such as the pancreas, testis, ovary, adrenal gland, pituitary gland and thyroid gland. The testis has the highest level of ACE2 expression, followed by the thyroid, whereas the hypothalamus has the lowest level[206].

The pituitary–thyroid axis should be regarded as a vulnerable target of SARS-CoV-2, and pituitary damage, whether direct or indirect, has been recognized as a determining factor of secondary hypothyroidism (functional or organic). As 3,5,3′-triiodothyronine (T3) and thyroxine (T4) are positively correlated with serum ACE levels, the ACE level could be used as a marker to investigate the action of peripheral thyroid hormones. A study by Rotondi et al. also proposed the thyroid as a potential target for SARS-CoV-2, as thyroid follicular cells encode the messenger RNA for ACE2 receptors[207].

After entering the body, SARS-CoV-2 may cause numerous clinical symptoms, SARS and multisystem organ failure by causing both direct and indirect injury to the body. The direct effect is due to the cytotoxic effect of the virus on the target cell, and the indirect effect is caused by the aberrant immune inflammatory responses, which include cytokine, complement systems and coagulation[208]. Innate and adaptive immune responses are regulated by thyroid hormones via genomic and nongenomic pathways. Cytokine production and release are triggered by T4 and T3; this results in a “cytokine storm”, which usually accompanies systemic viral infections.

Furthermore, thyroid hormones can enhance the antiviral activity of interferon-γ, thus explaining why some immune system pathways, such as cytokine and T helper 1 cell hyperactivation, occur in response to virus infections in thyroid disorders. It is also worth noting that T4 is capable of activating human platelets, which lead to pathological clotting, which is a complication of virus infection[209]

2.22 Effect of Vaccination on Thyroid Function

Viruses with associated inflammatory immune responses could be considered an important variable affecting lifelong thyroid function and consequently contribute to the definition of thyroid biography at the individual level[210]. The impact of viruses on thyroid function can undoubtedly lead to multisystem damage, as thyroid hormone affects the development and function of virtually all human cells, including neural maturation of olfactory receptor neurons. The vaccines approved to date are highly effective against severe disease. It is reassuring that although vaccine effectiveness against infection appears to decline with increasing time since vaccination, it continues to perform well against severe disease[211].

It is estimated that between 15% and 30% of hospitalized COVID-19 patients have thyroid dysfunction, however, most of these changes appear to be limited and that thyroid function in most patients will return to normal once the infection clears[212]. Nevertheless, there are two types of thyroid dysfunction that appear to be clearly associated with COVID-19 infection: Hypothyroidism due to non-thyroidal illness syndrome NTIS, which is changes in the levels of thyroid hormone in the blood observed in severely ill patients with the absence of pituitary and thyroid dysfunction. Among the possible SARS-CoV-2 vaccine complications, thyroid disease was not initially described, but early on, case reports of subacute thyroiditis, Graves' disease, and thyroid eye disease possible related to the COVID-19 vaccination began to

appear in the literature, and the question whether the thyroid disorder might be a SARS-CoV-2 vaccine complication has been raised[213].

2.23 Effect of SAR-CoV2 Vaccination on Thyroid Gland

For the COVID-19 pandemic, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) mRNA vaccines have been authorized and have provided durable benefits in reducing the risk of the severe outcomes of hospitalization and death[214]. To date, three doses of SARS-CoV-2 mRNA vaccination (the initial two doses and the third 'booster' dose) have been introduced in the many countries. As the fourth doses of the SARS-CoV-2 mRNA vaccines were only authorized for those older than 50 years in the US, the vaccination program is speculated to be continued specifically in the population who are at greatest risk and who might gain most benefit from vaccination. This includes immunocompromised individuals and people older than 50 years, given the prevalence of comorbidities that increase the risk of severe disease and death in such individuals[215].

Despite the robust beneficial data on the SARS-CoV-2 mRNA vaccine, adverse effects of the vaccines related to endocrine disorders have also recently been highlighted. Among the endocrine organs possibly targeted as adverse effects of the vaccine, the thyroid gland is the most common one[216]. Notably, an increasing number of cases of new-onset or relapse of Graves' disease (GD) has been recently reported to occur following the SARS-CoV-2 mRNA vaccines. Such case reports and series have raised speculation of the development of thyroid autoimmunity due to the mRNA vaccine. However, there is limited epidemiological evidence or basic findings to clarify if the SARS-CoV-2 mRNA vaccine induces thyroid autoimmunity[217]. Furthermore, if the mRNA vaccine disrupts thyroid autoimmunity, further clinically important questions arise: if the disruption has the potential to induce functionally overt situations, how long does the vaccine affect thyroid autoimmunity, and what factors predict its disruption?

There are similar concerns for COVID-19 vaccination in triggering thyroid autoimmunity and causing thyroid dysfunction. The earliest case report in May 2021 described 2 patients without known thyroid disorder who developed Graves' disease a few days after receiving the Pfizer-BioNTech messenger RNA (mRNA) COVID-19 vaccine[218]. Following these cases, there was a report of a patient who developed thyroiditis around 2 weeks after Pfizer-BioNTech mRNA COVID-19 vaccination. Since then, there have been more than 80 cases of thyroid dysfunction following COVID-19 vaccination. One postulated mechanism is "autoimmune/inflammatory syndrome induced by adjuvants" (ASIA). For example, the aluminum hydroxide as the adjuvant of CoronaVac may be the culprit inducing subacute thyroiditis as postvaccination ASIA. Molecular mimicry is another postulated mechanism: SARS-CoV-2 spike protein, nucleoprotein, and membrane protein all cross-react with thyroid peroxidase (TPO), suggesting that anti-SARS-CoV-2 antibodies may promote autoimmune thyroiditis[219]. Furthermore, differences exist in the target antigens and types and intensities of immune responses with different SARS-CoV-2 vaccines. BNT162b2 is the first mRNA vaccine widely used in humans. By stimulating dendritic cell maturation and eliciting robust T- and B-cell responses, RNA-based vaccines may activate bystander autoreactive lymphocytes. Hence, there is a theoretical concern that the mRNA vaccine may reactivate autoimmune diseases. The potential of different SARS-CoV-2 vaccines in altering thyroid function and autoimmunity remains to be elucidated[220].

2.24 Future Vaccination and Management

At the beginning of the COVID -19 pandemic, the majority of world authorities anticipated that herd immunity would be induced after approximately 67% of the population had been immunized[221]. However, the recent spread of the new SARS-CoV-2 variant among immunized individuals has necessitated a reconsidering of some previous assumptions. The expectation of severe disease prevention, and a reduction in hospitalization and mortality in

all vaccine recipients was altered by this realistic understanding of the effectiveness of vaccination[222]. Global access to COVID-19 vaccinations remains a priority, although some wealthy nations have access to an effective vaccination, this does not necessarily mean that everyone, particularly the poorest and most disadvantaged, will have this same access. Hence, to put an end to this epidemic, it will be critical that people in all countries, not just rich countries, receive the required protection by an effective vaccine. Recently, the world population had received at least one dose of a COVID-19 vaccination[223].

According to research conducted around the world, factors preventing vaccination include concerns about safety and side-effects of the vaccine, effectiveness, lack of trust, misinformation, and anti-vaccine campaigns. The most significant concern expressed by Europeans who were unsure about getting vaccinated was potential side effects[224]. These studies indicated that different factors contribute to vaccine hesitancy in different countries, and that there is a need to define a more in-depth understanding of why some individuals are hesitant to participate in the vaccination program[225]. This points to the necessity for a tailored approach to vaccine communication, and in particular, a differentiated reaction to vaccination hesitancy in different communities, in order to fulfill global herd immunity targets. To put an end to the global SARS-CoV-2 pandemic, a vaccine is required. Any vaccine that is dependable, effective, long-lasting, and widely available is a suitable choice. However, viral particles might mutate, rendering vaccines ineffective[226].

Therefore, it is critical to develop a safe and dependable vaccine in advance for future outbreaks of SARS-CoV-2 variants[227]. Regarding the nature of the virus and high rate of RNA change with natural replication that caused varying transmissibility in pathogenicity of unlimited variants, as well as ongoing concern about antigenic changes affecting vaccine protection, it is critical to recognize that it is not possible to completely eradicate this virus from the world, there should be a developed long-term plans for dealing with it after the

control of future disease surges and mortality by available vaccines. In addition to decreasing antiviral antibodies, there should be a repeated vaccine at unpredicted intervals due to the antigen diversity of newly emerging subspecies[228,229].

Endnotes

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Chapter Three

Methodology

3.1 Study Design

This study was a cross- sectional study comprising of one hundred and thirty nine individuals (from sample size calculation) who had been vaccinated at most eight months prior to sample analyses and tested negative to SARS-CoV-2 infection from the result of Real–Time- polymerase chain reaction (RT-PCR) method presented and vaccination record. These individuals were compared with a matched cohort of one hundred and forty five apparently healthy individuals, unvaccinated and not positive to SARS-CoV-2 infection from the result of Real–Time- polymerase chain reaction (RT-PCR) method presented. Individuals with previous history of thyroid dysfunction, diabetes, hypertension and renal dysfunction were excluded from the study. The above mentioned participants were further screened down by analysis of SARS-CoV-2 antibodies to ascertain those that were truly positive and negative. The study was conducted for a period of six months.

3.2 Ethical Considerations

Ethical approval was obtained from the Oyo State Ethical Review Committee. Informed consent was obtained before recruiting anyone for the study.

3.3 Study Area

This study was conducted in some Local Governments areas (LGAs) in Oyo state (Ibadan North, Ibadan South East and Oluyole). Individuals who had been vaccinated were recruited randomly as evident in their vaccination cards from Government Hospitals within Ibadan,

Oyo State after informed consent had been obtained while unvaccinated group were recruited upon seeing the RT-PCR result as negative from Government testing sites within the state, after informed consent had been given.

3.4 Study Population

3.4.1 Vaccinated Participants (Cases)

A total of one hundred and thirty nine apparently healthy participants (males and females) age range 18-60 years who had been vaccinated as documented in the SARS-CoV-2 vaccination cards and tested negative to SARS-CoV-2 infection from the result of RT-PCR method presented were recruited across some Local Government arears in Oyo State.. The cases were then screened down to eighty two during analysis by excluding those with negative Sars-CoV-2 IgG results (to ascertain those who are truly vaccinated), elevated blood pressure > 130/85mmHg, raised HBA1c >5.8%, serum Urea > 45mg/dl and creatinine > 1.5mg/dl. (See Figure 3.1: study flow chart for cases)

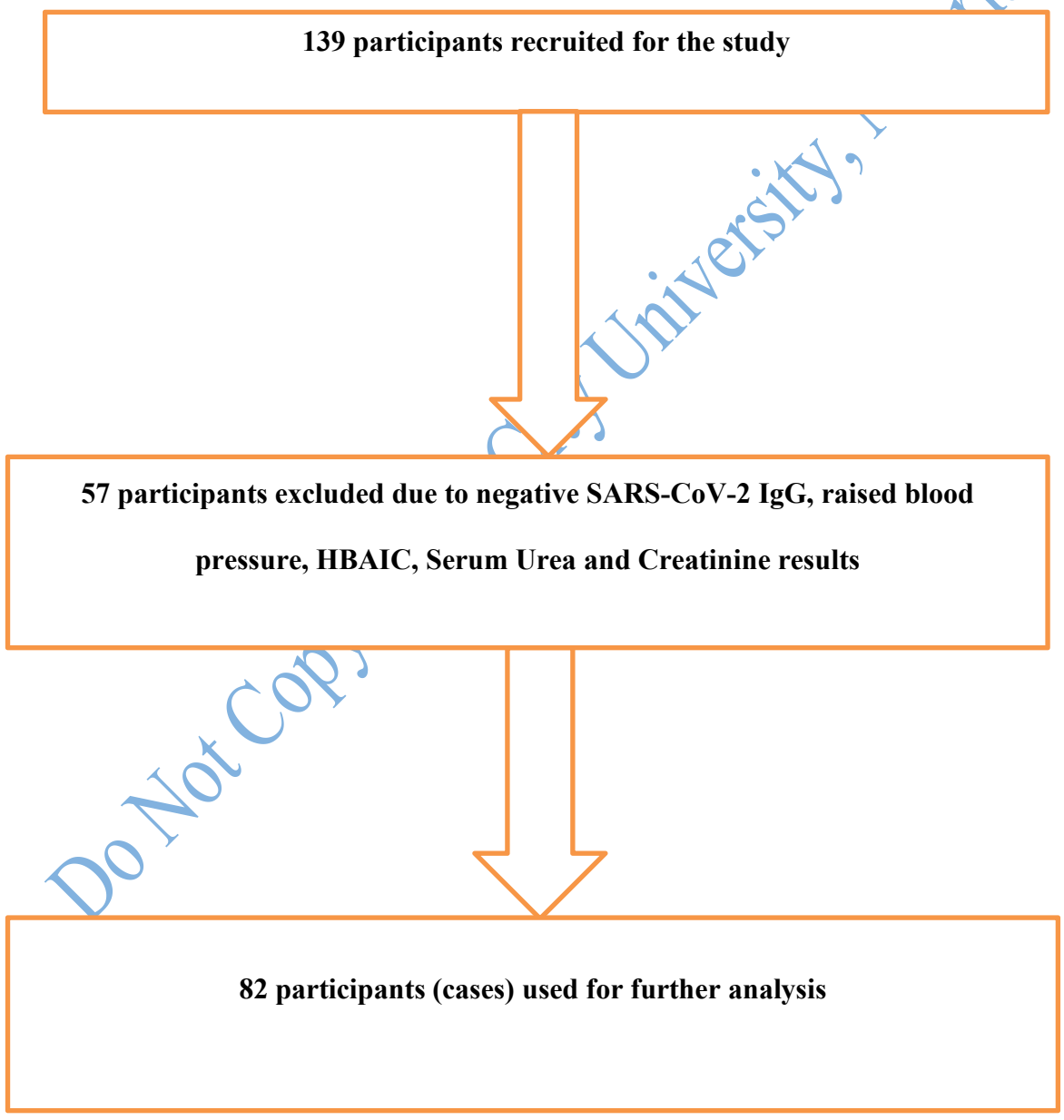

Fig. 3.1 Study flow chart for Cases

Source: Author's Field Work, 2023

3.4.2 Unvaccinated Participants (Controls)

A total of one hundred and forty five apparently healthy participants (males and females) age range 18-60 years from different Local Government arears in Ibadan, not vaccinated and tested negative to SARS-CoV-2 infection using RT-PCR result as documented from the Government testing sites were recruited after informed consent had been obtained. These controls were then screened down to eighty six during analysis by excluding those with positive Sars-CoV-2 IgG results (to ascertain those that are truly negative or unexposed), elevated blood pressure > 130/85mmHg, raised HBA1c >5.8%, serum Urea > 45mg/dl and creatinine > 1.5mg/dl. (See Figure 3.2: study flow chart for controls)

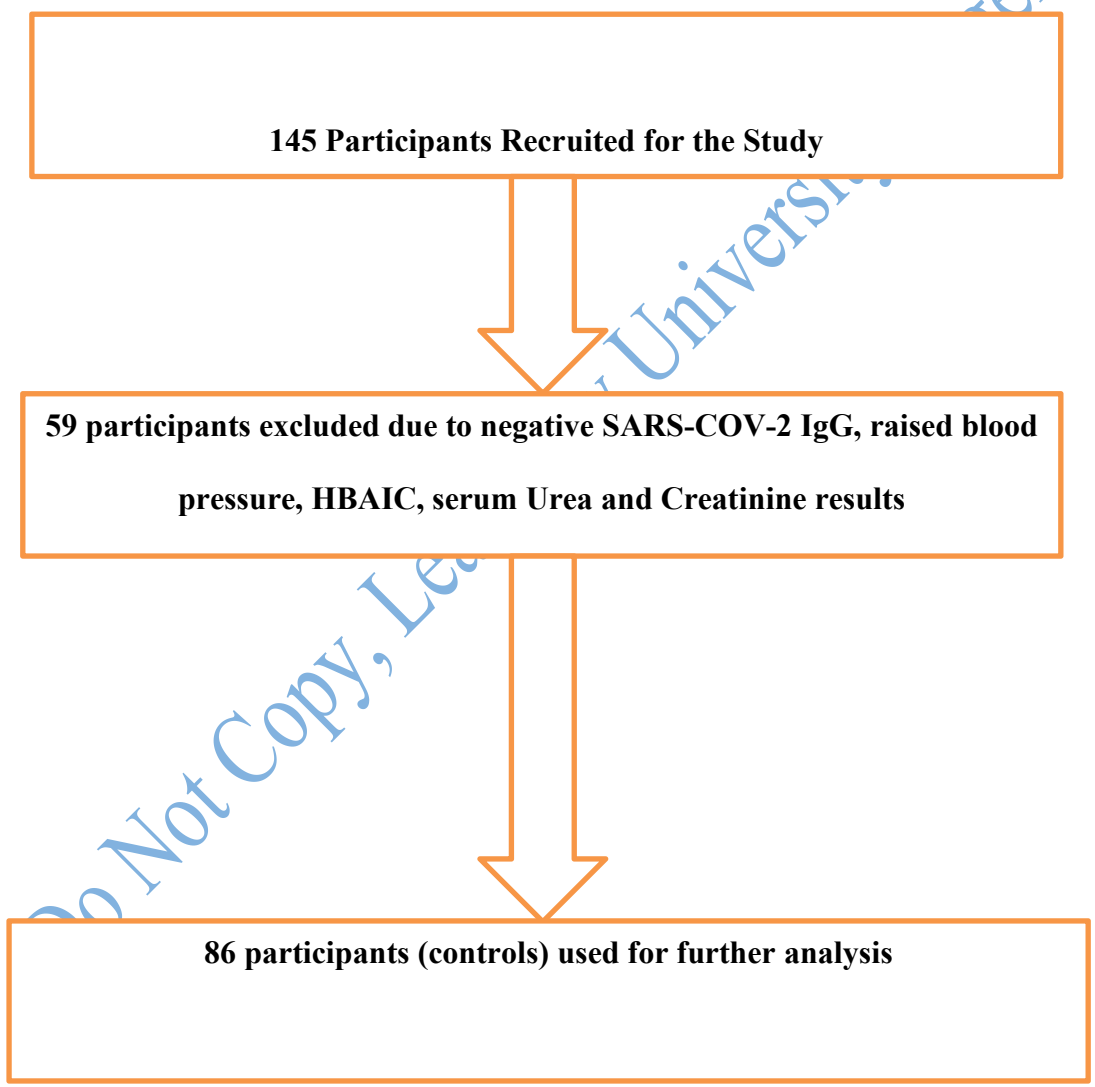

Fig. 3.2 Study flow chart for Controls

Source: Author's Field Work, 2023

3.5 Sample Size Determination

The formula for sample size calculation for descriptive cross- sectional study is: $N = \frac{Z^2pq}{d^2}$

Where:

Zα = standard normal deviate corresponding to 2 sided level of significance at 5% = 1.96

P = prevalence of SARS-CoV-2

q = 1- prevalence

d= level of precision at 5% = 0.05

The prevalence of SARS-CoV-2 in Nigeria is 5.6% in Nigeria (Zhu et al., 2020).

P = 5.6% = 0.056

N = Number of participants needed

q= 1- 0.056 = 0.94

$N= \frac{(1.96)^2 X0.056X\ 0.94}{(0.05)^2} = 80.88$

Therefore, the minimum sample size required is 81 for each of the group.

3.6 Sampling Strategy: Convenience sampling strategy was employed during this research. Structured questionnaire was used to obtain the socio demographic history of all the participants.

3.7 Selection Criteria

2.7.1 Inclusion Criteria for Cases:

- Apparently healthy individuals age range 18-60 years who had received the complete dose of Sars-CoV-2 vaccines (presence of SARS-CoV-2 antibodies). The records of such were gotten from the vaccination card and documented in the questionnaire administered.

2.7.2 Inclusion Criteria for Controls

- Apparently healthy individuals age range 18- 60 years, who had never tested positive nor vaccinated (absence of SARS-CoV-2 antibodies) were recruited for this study from the RT- PCR testing sites

2.7.3 Exclusion Criteria for the Cases

- Individuals outside of the age range, on medication and with history of hypertension, diabetes mellitus (DM) and cardiovascular diseases (CVD), thyroid dysfunction
- Refusal to give consent

2.7.4 Exclusion Criteria for the Controls

- Individuals on medications that could affect the thyroid hormones
- Individuals with history of hypertension, diabetes mellitus (DM) and cardiovascular diseases (CVD)
- Refusal to give consent

3.8 Data Collection Procedure

3.8.1 Demographic Indices

A semi structured pre-test questionnaire was administered to obtain qualitative data about the age, gender, background, socio- economic status and the dietary life style of the participants

3.8.2 Anthropometric Characteristics:

Physical indices (weight, height, body mass index) were obtained from the participants

3.8.2.1 Weight:

This was taken with a bathroom scale placed on a flat surface. The participants wore light clothing and without shoes and stood on the zero scale. The reading was recorded in duplicates to the nearest 0.5kilogram (kg) and the average reading was taken

3.8.2.2 Height:

The height of each participant was measured in meters with subjects standing bare footed as upright as possible on a hard level ground against a vertical wall and without raising the heels from the ground with the feet kept together while the back and heel were aligned with a ruled bar against the vertical surface. The measurements was made by moving a slide head piece to the vertex of the subject's head and the reading at that point was recorded to the nearest 0.1meter.

3.8.2.3 Body Mass Index (BMI):

This was calculated from the body weight and height of the subjects using the formula stated below:

$$BMI = \frac{\text{weight (kg)}}{\text{height}^2\ (m^2)}$$

3.9 Clinical Indices

Blood pressure (systolic and diastolic), measurement was taken from participants.

3.9.1 Blood Pressure

Principle

This involves auscultation of the brachial artery by the use of mercury sphygmomanometer. The rot cuff is tied around the forearm and is inflated to obstruct the brachial artery and a stethoscope is placed at the cubital fossa and the pressure released. As the blood flows through the arm the first and the second sound produced is the Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP) respectively.

3.10 Sample Collection

10mls of venous blood sample was aseptically obtained by venepuncture from the participants. The blood was dispensed into plain serum tubes and ethylenediamine tetraacetic acid (EDTA) bottles. The bloods in the plain bottles were kept for 1-2 hours to clot and serum obtained was stored at -20^{0}C until analysis. On the other hand, the EDTA blood whole was for used for glycated haemoglobin assay (HBA1C) after which the plasma from the EDTA was used for Covid-19 antibody assay. All clotted blood and EDTA samples (after HBA1C analysis were centrifuged at 4000rpm for 10 minutes using Centaur centrifuge, after which serum and plasma were extracted accordingly and stored in small aliquots at – 20^{0}C until analysis.

Serum obtained was used for thyroid function assay (tri-iodo-thyronine or free T3 (FT3), thyroxine or free T4 (FT4) and thyroid stimulating hormone (TSH), inflammatory marker or C- reactive protein (CRP) estimation, Kidney function tests (electrolytes (sodium, potassium, chloride and bicarbonate), urea, creatinine and cystatin C).

3.11 Laboratory Analyses

The biochemical indices that were analyzed were:

- Diabetic studies- HBA1C,

- COVID-19 antibodies.
- Thyroid function tests -Thyroid Stimulating Hormone (TSH), Tri-iodo- thyronine (fT3), Thyroxine (fT4)
- Inflammatory marker- C-reactive protein (CRP)
- Renal Function tests- Sodium, Potassium, Chloride, Bicarbonate, urea, creatinine and cystatin C

3.11.1 Determination of Biochemical Parameters

3.11.1. 2 COVID-19 Antibodies Estimation

Method: The plasma COVID-19 antibodies estimation was carried out on Architect I1000 (Abbot) auto- analyser using chemiluminescent microparticle immunoassay (CMIA) used for the qualitative detection of IgG antibodies to SARS-CoV-2 in human serum and plasma.

Principle: This assay is an automated, two-step immunoassay for the qualitative detection of IgG antibodies to SARS-CoV-2 in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA) technology[1].

- Sample, SARS-CoV-2 antigen coated paramagnetic microparticles, and assay diluent are combined and incubated. The IgG antibodies to SARS-CoV-2 present in the sample bind to the SARS-CoV-2 antigen coated microparticles.
- The mixture is washed. Anti-human IgG acridinium-labeled conjugate is added to create a reaction mixture and incubated.
- Following a wash cycle, Pre-Trigger and Trigger Solutions are added. The resulting chemiluminescent reaction is measured as a relative light unit (RLU).
- The presence or absence of IgG antibodies to SARS-CoV-2 in the sample is determined by comparing the chemiluminescent RLU in the reaction to the calibrator RLU, which is calculated by the system as an Index (S/C).

3.11.1.3 Determination of thyroid function markers

Thyroid Stimulating Hormone (TSH) Assay

Method: The serum TSH assay was carried out on Architect I1000 (Abbot) auto- analyser using the Chemiluminescent Microparticle Immunoassay for the quantitative determination of Thyroid Stimulating Hormone in human serum or plasma

Principle: The Architect TSH assay is a two-step immunoassay to determine the presence of TSH in human serum and plasma using Chemiluminescent Microparticle Immunoassay (CMIA) technology with flexible assay protocols referred to as Chemiflex[8].

- The sample, anti- β TSH antibody coated paramagnetic microparticles and TSH assay diluent are combined. TSH present in the sample binds to the anti- TSH antibody coat microparticles.
- After washing, anti-α TSH acridinium- labelled conjugate is added to create a reaction mixture.
- Following another wash cycle, Pre-trigger and Trigger solutions are added to the reaction mixture.
- The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is a direct relationship between the amount of TSH in the sample and the RLUs detected by the Architect I System optics

Free T3 Assay

Method: The serum fT3 assay was carried out on Architect I1000 (Abbot) auto- analyser using the Chemiluminescent Microparticle Immunoassay for the quantitative determination of free triiodothyronine in human serum or plasma

Principle: The Architect fT3 assay is a two-step immunoassay to determine the presence of free triiodothyronine (unbound T3) in human serum and plasma using Chemiluminescent Microparticle Immunoassay (CMIA) technology with flexible assay protocols referred to as Chemiflex[8].

- Sample and anti- T3 coated paramagnetic microparticles are combined. Free T3 present in the sample binds to the anti-T3 coated microparticles.
- After washing, T3 acridinium- labelled conjugate is added.
- Pre- trigger and Trigger solutions are then added to the reaction mixture.
- The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is an inverse relationship between the amount of fT3 in the sample and the RLUs detected by the Architect I System optics.

Free T4 Assay

Method: The serum fT4 assay was carried out on Architect I1000 (Abbot) auto- analyser using the Chemiluminescent Microparticle Immunoassay for the quantitative determination of free thyroxine in human serum or plasma

Principle: The Architect fT4 assay is a two-step immunoassay to determine the presence of free thyroxine (Unbound T4) in human serum and plasma using Chemiluminescent Microparticle Immunoassay (CMIA) technology with flexible assay protocols referred to as Chemiflex[8].

- Sample and anti- T4 coated paramagnetic microparticles are combined. Free T4 present in the sample binds to the anti-T4 coated microparticles.

- After washing, T4 acridinium- labelled conjugate is added to create a reaction mixture.
- Following another wash cycle, Pre- trigger and Trigger solutions are then added to the reaction mixture.
- The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is an inverse relationship between the amount of fT4 in the sample and the RLUs detected by the Architect I System optics

3.11.1.4 Determination of Inflammatory marker – CRP

Method: The estimation of CRP was carried out on Architect c4000 using the Quantitative Immunoturbidimetric method

Principle: CRP is an acute phase protein whose concentration rises non-specifically in response to inflammation. Multigent CRP vario is a latex immunoassay developed to accurately and reproducibly measure blood CRP levels in serum and plasma[2].

When an antigen-antibody reaction occurs between CRP in a sample and anti-CRP antibody, which has been adsorbed to latex particles, agglutination results. This agglutination is detected as an absorbance change at 572nm, with the rate of change being proportional to the quantity of CRP in the sample.

3.11.1.5 Renal Function Tests

Determination of Serum Sodium, Potassium and Chloride

Methodology: The ARCHITECT c4000System's Integrated Chip Technology (ICT) was used for the quantitation of sodium, potassium, and chloride in human serum by adopting the principle ofIon-selective electrode diluted (Indirect) on Architect c4000

Principle: Ion-selective electrodes for sodium, potassium, and chloride utilize membranes selective to each of these ions. An electrical potential (voltage) was developed across the membranes between the reference and measuring electrodes in accordance with the Nernst

equation. The voltage was compared to previously determined calibrator voltages and converted into ion concentration[3].

Determination of Serum Bicarbonate

Method: Phosphor Enol Pyruvate (PEP) Carboxylase

Principle: Carbon dioxide, as bicarbonate (HCO_3^-), and phosphor (enol) pyruvate (PEP) are converted to oxalacetate and phosphate in the reaction catalyzed by phosphor (enol) pyruvate carboxylase (PEPC). Malate dehydrogenase (MDH) catalyzes the reduction of oxalacetate to malate with the concomitant oxidation of reduced nicotinamide adenine dinucleotide (NADH) analog. The resulting decrease in absorbance at 404 nm is proportional to the carbondioxide (CO_2) content in the sample[6].

Determination of Serum Urea Nitrogen

Method: The urea assay quantification was carried out on Archiect c4000 auto analyser using the urease method

Principle: The Urea Nitrogen assay is a modification of a totally enzymatic procedure. The test was performed as a kinetic assay in which the initial rate of the reaction is linear for a limited period of time. Urea in the sample was hydrolyzed by urease to ammonia and carbon dioxide. The second reaction, catalyzed by glutamate dehydrogenase (GLDH), converts ammonia and α-ketoglutarate to glutamate and water with the concurrent oxidation of reduced nicotinamide adenine dinucleotide (NADH) to nicotinamide adenine dinucleotide (NAD). Two moles of NADH were oxidized for each mole of urea present. The initial rate of decrease in absorbance at 340 nm is proportional to the urea concentration in the sample[4].

Determination of Serum Creatinine

Method: The creatinine assay quantification was carried out on Archiect c4000 auto analyser using the Kinetic Alkaline Picrate method

Principle: At an alkaline pH, creatinine in the sample reacts with picrate to form a creatinine-picrate complex. The rate of increase in absorbance at 500 nm due to the formation of this complex is directly proportional to the concentration of creatinine in the sample[5].

Determination of serum Cystatin-C

Method: Estimation of Cystatin C was carried out on Roche/ Cobas c311 auto analyser using the immunoturbidimetric assay method.

Principle: Particle enhanced immunoturbidimetric assay Human cystatin C agglutinates with latex particles coated with anti-cystatin C antibodies. The aggregate is determined turbid-metrically at 552 nm[9].

3.11.1.6 Determination of Hemoglobin A1C

Method: The quantification of HBA1C was carried out on Architect c4000 using the Enzymatic method.

Principle: The Hemoglobin A1c assay consists of two separate concentration measurements: glycated hemoglobin (HbA1c) and total hemoglobin (THb). The two concentrations are used to determine the percent HbA1c (NGSP units) or the hemoglobin fraction in mmol/mol (IFCC units)[7].

The individual concentration values of HbA1c and THb generated by the Hemoglobin A1c assay are used only for calculating the percent hemoglobin A1c or HbA1c fraction, and must not be used individually for diagnostic purposes.

The anticoagulated whole blood specimen is lysed automatically on the system for the Whole Blood application or may be lysed manually using the Hemoglobin A1c Diluent (A1cDIL) for the Hemolysate application.

Glycated Hemoglobin (HbA1c)

The Hemoglobin A1c assay utilizes an enzymatic method that specifically measures *N*-terminal fructosyl dipeptides of the *β*-chain of HbA1c.

• In the pretreatment process, the erythrocytes are lysed and the hemoglobin is transformed to methemoglobin by reaction with sodium nitrite.

• With the addition of Reagent 1 (R1) to the sample, the glycosylated *N*-terminal dipeptide (fructosyl-VH) of the *β*-chain of hemoglobin is cleaved by the action of protease. The hemoglobin is transformed to stable methemoglobin azide by the action of sodium azide and the concentration of the hemoglobin is determined by measuring absorbance.

• Addition of Reagent 2 (R2) starts a reaction and fructosyl peptide oxidase (FPOX) is allowed to react with fructosyl-VH. The HbA1c concentration is measured by determining the resultant hydrogen peroxide.

Total Hemoglobin (THb)

The hemoglobin is oxidized to stable methemoglobin azide by the action of sodium nitrite and sodium azide and the concentration of the hemoglobin is determined by measuring absorbance (sample + R1).

Hemoglobin A1c Calculations

The final result is expressed as %HbA1c (NGSP) or mmol/mol HbA1c (IFCC) and is automatically calculated by the system from the HbA1c/THb ratio as follows:

mmol/mol HbA1c IFCC:

HbA1c (mmol/mol) = (HbA1c/THb) × 1000

%HbA1c DCCT/NGSP:

$$HbA1c\ (\%) = IFCC \times 0.09148 + 2.152$$

3.12 Statement of Confidentiality

All information and data collected in this study were given code numbers and the names of participants were not recorded. The report of this research cannot be linked to any of the participants in anyway and their names or any identifier will not be used in any publication.

3.13 Ethical Considerations

The ethical approval for this research study was obtained from the Oyo State Institutional Review Committee (Ethics Committee) being the State where the research work was carried out.

Confidentiality: All information and data collected in this study was given code numbers and the names of participants were not recorded. The report of this research cannot be linked to any of the participants in anyway and the name(s) or any identifiers will not be used in any publication.

Translation of protocol to participants in local language: The informed consent form and the questionnaires for this research study were translated to our local language (i.e. Yoruba language) for easy communication and proper understanding. The administration of the questionnaires was interactive and interviewer-administered.

Beneficence: The participants will become aware of the level of their thyroid and kidney function tests at the end of this study. No incentive were given to the participants, the study is for academic purpose

Non-Maleficence: Participation in this research posed no pain or danger or risk on the participants. It is only a little pain of needle prick for blood collections, few minutes (about 25minutes) for the filling of the questionnaires. All participants were treated with respect.

Voluntariness: Participation in the research was entirely voluntary.

3.13 Data Analysis

Data generated was analyzed using IBM SPSS (version 20.0). Results were presented as mean ± standard deviation for all parameters. Independent Student's t-test was used to evaluate significant differences between mean values. Pearson's correlation coefficient was used to examine association between quantitative variables. All P- values less than 0.05 ($p<0.05$) was considered statistically significant.

Endnotes

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Chapter Four

Results and Discussion of Findings

4.1 Cases and Controls

A total of two hundred and eighty-four (284) apparently healthy individuals were recruited for this study. Of this number, one hundred and thirty nine (139) individuals (males and females), vaccinated at most eight months prior to sample analyses were recruited as Cases, while one hundred and forty five (145) who were not vaccinated were recruited as Controls.

Cases and Controls were screened down to eighty-two (82) and eighty six (86) participants respectively. The eighty-two cases were selected by excluding fifty seven (57) individuals with negative Sars-CoV-2 IgG results, elevated blood pressure (> 130/90mmHg), raised HBA1C values (>5.7%), raised serum urea (>45mg/dl) and creatinine (> 1.5mg/dl). While the eighty six participants recruited as Controls were selected by excluding fifty nine (59) individuals with positive Sars-CoV-2 IgG results, elevated blood pressure (> 130/90mmHg), raised HBA1C (>5.7%) values, raised serum urea (>45mg/dl) and creatinine (> 1.5mg/dl). The eighty-two cases and eighty six controls were used for further testing in this work.

The screening of the participants was done to remove bias and cofounders that could affect the interpretation of the results subsequently. Thus ensuring that the cases were truly vaccinated and the controls were neither exposed nor vaccinated.

4.2 Demographic Data of All the Participants

Table 4.1 shows the bio data and socio demographic characteristics) of the eighty-two vaccinated participants (cases). The age range was between 18 and 60 years, comprising of forty-nine males and thirty-three females. Most of these participants were married and having 40.2% within the age group of 26-40 years. Over 90% resides in Ibadan North local Government area. The cases were vaccinated with three known types of vaccines, Pfizer (33%), Moderna (3.3%) and Astrazeneca (11%). However, 52.7% received two different types of vaccines to complete their dose. The SARS-CoV-2 IgG antibodies results of cases were all confirmed as positive before proceeding with further analysis.

In addition, 59.3% had their BMI within the normal range of 18.6- 24.9kg/m^2, while 31.4% were overweight (BMI between 25- 29.9kg/m^2) and the rest 3.5% were underweight. (BMI <18.5kg/m^2).

Table 4.2 shows the mean ± SD of anthropometric measurements and blood pressure of cases. The mean blood pressure (BP) was 124.5/80.3mm/Hg, while the mean BMI was 23.8kg/m^2. The overall values of both BP and BMI are keeping well with the reference value of 90/60 - <130/80mmHg and 18.5- 24.9kg/m^2 respectively. The mean ± SD of blood pressure and BMI variables for the excluded cases are at the appendix.

Table 4.1: The Biodata and Socio Demographic Characteristics of Cases

Variables	n (%)
Age Group (years)	
19-25	19 (23.1)
26-40	**33 (40.2)**
41-60	30 (36.6)
Sex	
Female	33 (40.2)
Male	**49 (59.8)**
Place Of Residence	
Ibadan North	**74 (90.1)**
Oluyole	4 (4.4)
South East	4 (5.5)
Marital Status	
Divorcee	1 (1.1)
Married	**44 (53.8)**
Single	37 (45.1)
Vaccination	
Astrazeneca	10 (11.0)
Pffizer	**30 (33.0)**
Moderna	3 (3.3)
Combined	48 (52.7)
BMI (kg/m^2)	
<18.5	3 (3.5)
18.6-24.9	**51 (59.3)**
25-29.9	27 (31.4)

n- Number of occurrence or frequency, %- percentage frequency, BMI- Vaccinated apparently healthy participants.

Source: Author's Field Work, 2023

Table 4.2: The Anthropometric Measurements and Blood Pressure of Cases

Variables	Mean ± SD
Blood Pressure	
Systolic BP (mmHg)	124.5 ± 6.9
Diastolic (mmHg)	80.3 ± 7.9
Weight (Kg)	68.6 ± 9.4
Height (cm)	169 ± 7.1
BMI (Kg/m^2)	24.0 ± 3.6
HBA1C (%)	5.0 ± 0.4

S.D- Standard Deviation, BMI- Body mass index, BP- Blood pressure, HBA1C- Glycated hemoglobin. The results are expressed as Mean ± SD.

Source: Author's Field Work, 2023

Table 4.3 summarizes the bio data and socio demographic characteristics of the unvaccinated participants (controls). The age range between was between 18 and 60 years, comprising of fifty-eight males and twenty-eight females. Most of these participants were married and within the age group of 26-40 years. Over 50% resides in Ibadan North local Government area. In this category of participants, 59.3% had BMI within the reference age, 31.4% were overwight, 5.8% were obese and the rest 3.5% were underweight. None of them were vaccinated. The SARS-CoV-2 IgG antibodies were negative, thus confirming that they had not been exposed or vaccinated before this study.

Table 4.4 shows the mean ± SD of anthropometric measurements and blood pressure of controls. The mean blood pressure was 124.6/80.4mm/Hg, while the mean BMI was 24.0.kg/m^2 Both BP and BMI are keeping well with the reference values of 90/60 - <130/80mmHg and 18.5- 24.9kg/m^2 respectively. The mean ± SD of the excluded controls had been moved to the appendix.

Table 4.3: The Biodata and Socio Demographic Characteristics of the Controls

Variables	n (%)
Age Group (years)	
19-25	18 (20.9)
26-40	**52 (60.5)**
41-60	16 (15.0)
Sex	
Female	28 (32.6)
Male	**58 (67.4)**
Place Of Residence	
Ibadan North	55 (64.0)
Oluyole	29 (23.3)
South east	11 (12.7)
Marital Status	
Divorced	3 (4.4)
Married	**60 (69.8)**
Single	23 (26.7)
Vaccination	**Unvaccinated**
BMI	
<18.5	3 (3.5)
18.6-24.9	**51 (59.3)**
25-29.9	27 (31.4)
>30.0	5 (5.8)

n- Number of occurrence or frequency, %- percentage frequency, Controls- Unvaccinated apparently healthy participants, BMI- Body mass index

Source: Author's Field Work, 2023

Table 4.4: The Anthropometric Measurements and Blood Pressure of Control.

Variables	Mean ± SD
Blood Pressure	
Systolic BP (mmHg)	124.5 ± 7.9
Diastolic (mmHg)	80.4 ± 9.4
Weight (Kg)	68.6 ± 9.7
Height (cm)	169.2 ± 7.0
BMI (Kg/m^2)	23.8 ± 3.2
HBA1C (%)	5.2 ± 0.5

S.D- Standard Deviation, Controls- Unvaccinated apparently healthy participants. The results are expressed as Mean ± SD.

Source: Author's Field Work, 2023

4.3 Presentation of Laboratory Data for All Participants

Table 4.5 and 4.6 show the mean ± SD of the renal function, comprising of the electrolytes, urea, creatinine, cystatin C; the thyroid function tests which are FT3, FT4, TSH and inflammatory marker, CRP of the cases and controls respectively. All these parameters were within the reference ranges in both groups. The mean Sodium for cases and controls were 139.5 and 139.2mmol/l. These values were within the reference value of 135-145mmol/l. The Potassium values for the two groups were 4.0 and 3.9, which is within the acceptable refence value of 3.5 -5.0mmol/l. The Bicarbonate values for all the participants from the two groups were within the expected value of 20-30mmol/l. Chloride results for the cases and controls were 101.6 and 101.2mmol/l respectively, values that are within the acceptable range of 95-110mmol/l. These electrolyte values showed that the renal functions of both cases and controls as concerns electrolyte exchanges is adequate.

Furthermore, the mean urea (19.9mg/dl for both groups) and creatinine (0.8mg/dl of both groups), values of both the cases and controls, are within the reference value of urea: 15-45mg/dl and creatinine: 0.5-1.5mg/dl. This shows that none of the participants from the two-group had renal or thyroid dysfunction. The mean result of the predictive marker of renal function, cystatin C for both groups were 0.8 and 0.7mg/dl respectively (see Table 4.5), this is within the acceptable range of cystatin C (0.6-1.2mg/dl). The predictive marker, cystatin C is a better indicator of early renal dysfunction than the conventional urea and creatinine, thus the findings shows that the participants in both groups are not having any renal dysfunction.

In addition, Table 4.6 shows the mean values of the thyroid function hormones, FT3, FT4 and TSH. All the values were within the reference values for healthy individuals as shown in Table 4.6. This goes to show that the participants in the two groups were not having any form of thyroid dysfunction ranging from hypothyroidism, hyperthyroidism, sub-acute thyroiditis

or sub clinical thyrotoxicosis. The mean CRP value for the groups were also within the reference value for apparently healthy individuals, thus the two groups were not having any inflammatory reactions.

Table 4.7 shows the comparison of mean of the renal function, thyroid function and inflammatory markers between the cases and controls. The difference in mean values of these markers from cases and controls were not statistically significant ($p>0.05$). These findings indicated that the values of the renal and thyroid function parameters in the cases and controls were similar and there was no statistically significant difference in the two groups.

Table 8 shows the percentage of cases with elevated levels of the inflammatory and thyroid markers when compared with reference values. Only one unvaccinated participants (1.2% of cases) had elevated level of FT4, TSH and CRP than reference values. The total prevalence of elevated inflammatory and thyroid markers in cases was 3.6%. However, the markers were only minimally elevated but not statistically significant. This is indicative of sero prevalence of elevated levels of the thyroid and the inflammatory marker (CRP) in cases.

Table 9 shows the percentage of cases with elevated levels of kidney function and predictive kidney injury markers. None of these markers were elevated in cases. All parameters were within the reference values. The finding of normal levels of all the renal function parameters in cases is indicative of adequate renal function in these individuals.

Table 4.5: The Renal Function Markers and Predictive Marker of Renal Dysfunction in Cases and controls.

Variables	Cases (n= 82) Mean ± (SD)	Controls (n= 86) Mean ± (SD)
Renal Function Markers		
Sodium (mmol/L)	139.5 ± 2.0	139.2 ± 2.6
Potassium (mmol/L)	4.0 ± 0.2	3.9 ± 0.2
Chloride (mmol/L)	101.6 ± 2.9	101.2 ± 3.6
Bicarbonate (mmol/L)	24.0 ± 3.0	23.9 ± 1.7
Urea (mg/dL)	19.9 ± 6.5	19.9 ± 5.8
Creatinine (mg/dL)	0.8 ± 0.2	0.8 ± 0.2
Predictive Marker		
Cystatin C (mg/dL)	0.8 ± 0.2	0.7± 0.2

Values are in Mean ± SD, SD- standard deviation, Cases- Vaccinated apparently healthy participants, Controls- Unvaccinated apparently healthy participants. The results are expressed as Mean ± SD.

Source: Author's Laboratory

Table 4.6: Thyroid Function and Inflammatory Markers in Cases and Controls

Variables	Cases (n=82) Mean ± (SD	Controls (n=86) Mean ± (SD)
Thyroid Function Markers		
FT3 (Free triiodothyronine) pmol/L	3.9 ± 0.5	3.8 ± 0.4
FT4 (Thyronine) pmol/L	14.5 ± 2.5	15.1 ± 2.9
TSH (Thyroid stimulating Hormone) pmol/L	1.6 ± 0.9	1.5 ± 0.9
Inflammatory Marker		
C-Reactive Protein (mg/L)	0.7 ± 0.8	1.4 ±1.7

Values are in Mean ± SD, SD- standard deviation, Cases- Vaccinated apparently healthy participants, Controls- Unvaccinated apparently healthy participants. The results are expressed as Mean ± SD.

Source: Author's Laboratory Work, 2023

Table 4.7: Comparison of Inflammatory Markers, Renal and Thyroid Function Test Between The Cases and Controls

Markers	Cases (n=82) Mean (SD)	Control (n= 80) Mean (SD)	t- value	P-value
Inflammatory Marker				
C- Reactive Protein (mg/dL)	0.7 (0.8)	1.4 (1.7)	-1.6	0.1
Renal Function Markers				
Sodium (mmol/L)	139.5 (2.0)	139 (2.6)	0.7	0.5
Potassium (mmol/L)	4.0 (0.2)	3.9 (0.2)	0.5	0.6
Chloride (mmol/L)	101.6 (2.9)	101.2 (3.6)	0.7	0.5
Bicarbonate (mmol/L)	24.0 (3.0)	23.9 (1.7)	0.4	0.7
Urea (mg/dL)	19.9 (6.5)	19.9 (5.8)	0.1	0.9
Creatinine (mg/dL)	0.8 (0.2)	0.8 (0.2)	1.6	0.1
Predictive markers				
Cystatin-C (mg/dL)	0.8 (0.2)	0.7 (0.2)	0.4	0.7
Thyroid Function Markers				
FT3 (pmol/L)	3.9 (0.5)	3.8 (0.4)	1.7	0.1
FT4 (pmol/L)	14.5 (2.5)	15.1 (2.9)	-1.3	0.2
TSH (pmol/L)	1.6 (0.9)	1.5 (1.0)	0.6	0.5

Values are in mean, $p<0.05$ is considered statistically significant, *- significance, p- probability, FT3- triiodothyronine, FT4- thyroxine, TSH- thyroid stimulating hormone, Cases- Vaccinated participants, Controls- Unvaccinated apparently healthy participants. The results are expressed as Mean ± SD.

Source: Author's Laboratory Work, 2023

Table 4.8: Prevalence of Renal Dysfunction in Cases

Markers	Caese (n=82) Mean ± SD	Reference Range	Number Low	Number Normal	Number Elevated	Percentage of Population with Elevated level
Sodium (mmol/L)	139.2 ± 2.6	135-145	3	79	0	0
Potassium (mmol/L)	4.0 ± 0.2	3.5-5.0	3	79	0	0
Chloride (mmol/L)	101.6 ± 2.9	95-110	3	79	0	0
Bicarbonate (mmol/)	24.0 ± 3.0	20-30	0	82	0	0
Urea (mg/dl)	19.9 ± 6.5	15-45	36	46	0	0
Creatinine (mg/dl)	0.8 ± 0 .2	0.5-1.5	6	76	0	0
Cystatin-C mg/L)	0.7 ± 0.2	0.6-1.5	11	71	0	0

Values are in Mean ± SD, SD- standard deviation, Cases- Vaccinated apparently healthy participants, Controls- Unvaccinated apparently healthy participants.

Source: Author's Laboratory Work, 2023

Table 4.9: Prevalence of Inflammation and Thyroid Dysfunction in Cases

Markers	Caese (n=82) Mean ± SD	Reference Range	Number Low	Number Normal	Number Elevated	Percentage of Population with Elevated level
Inflammatory Markers						
C- Reactive Protein (mg/L)	0.7 ± 0.8	<5.0	0	81	1	1.2
Thyroid Function Markers						
FT3 (pmol/L)	3.9 ± 0.5	3.35- 5.15	10	72	0	0
FT4 (pmol/L)	14.5 ± 2.5	10.6 – 21.0	0	81	1.2	1.2
TSH (pmol/L)	1.6 ± 0.9	0.38 – 4.31	0	81	1.2	1.2

Values are in Mean ± SD, SD- standard deviation, Cases- Vaccinated apparently healthy participants, Controls- Unvaccinated apparently healthy participants, FT3- triiodothyronine, FT4- thyroxine, TSH- thyroid stimulating hormone.

Source: Author's Laboratory Work, 2023

4.4 Discussion of Findings

Data from this study showed that the age of both cases and controls ranges between 18-60 years. The highest frequency being in participants whose ages are between 26-40 years from the two group. This finding is consistent with the previous study that this age group are more active, matured and well educated on the need for vaccination[3].

Information from this study showed that Pfizer vaccine was the most widely taken in cases (33%). This agrees with earlier studies that, mRNA vaccines are the most widely used vaccines and considered safer over other vaccine after its approval was given under emergency use authorization by the US Food and Drug Administration[4].

The serum levels of the inflammatory, renal and thyroid function markers of both cases and controls were within the reference range. The values of these biochemical parameters in cases showed no significant difference when compared with the Controls ($p>0.05$). This finding was contrary to earlier studies that reported less than 40% of newly diagnosed cases of glomerulonephritis and 30% cases of MCD following vaccination with mRNA vaccines[5].

Considering the cases reported in the earlier literatures, the onset of the symptoms of renal dysfunction, occurred as early as few hours after the first dose[6]. Hence, it is possible that some of the patients might have had signs of kidney injury (e.g., elevated creatinine, proteinuria, or microscopic hematuria) before the first dose but had not been medically evaluated before the commencement of the vaccination[7].

Few studies reported that most AKI developed within two weeks after immunizations[3]. However, a causal relationship between COVID-19 vaccinations and AKI cannot be established. It was not uncommon that AKI or nephropathies could be caused by various

vaccines, including flu, H1N1, and others[13]. Nonetheless, the incidence of COVID-19 vaccines related to AKI may be even higher, probably for the following reasons.

First, as mass vaccination continued, people at risk of AKI will receive the COVID-19 vaccine but most likely will not receive vaccines such as influenza. Second, the mRNA vaccine has been developed and refined for nearly two decades, but it was not widely used clinically until only recently[14]. Compared with previous vaccines, mRNA vaccine can elicit robust antibody- and cell-mediated immune responses, as demonstrated by its significantly higher neutralizing antibody titers and activated $CD4^+$ and $CD8^+$ T cells accompanied by increasing pro-inflammatory cytokines[16]. Moreover, it was noted that potential risks of cross reactivity between SARS-CoV-2 and autoimmune target proteins could lead to immune activation that may aggravate, unmask, or incite autoimmune diseases[23]. This results may also imply that mRNA vaccines (Pfizer-BioNTech and Moderna) were more commonly associated with AKI than adenovirus vaccines (Janssen).

Third, the immune response to the COVID-19 vaccine may mimic what happens in response to natural infection. The kidneys are not excluded during the involvement of COVID-19 disease, there are several renal dysfunction cases in COVID-19 infection. Up to 50% of hospitalized patients with COVID-19 presented AKI and higher mortality, especially critically ill patients (40–60%)[2]. The underlying mechanisms of COVID-19-associated AKI are likely multifactorial, including local and systemic inflammatory and immune responses, endothelial injury and hypercoagulability, and the renin-angiotensin system, direct viral infection[10].

In addition, IgA nephropathy is the most common glomerulonephritis identified in kidney biopsy, and it is an immune complex disease caused by mesangial IgA1 deposition with or without concurrent IgG and C3 deposits [17]. Although the factors causing the occurrence of

IgA nephropathy have not been clearly identified, IgA nephropathy is proposed as a multi-hit disease. If patients who have a genetic predisposition (genetic variation encoding galactosylation) are exposed to subsequent triggering events, such as infection, dietary and environmental stress lead to the production of anti-glycan IgA/IgG, and IgA nephropathy will occur[8]. It was reported in an earlier study that COVID-19 vaccination was associated with glomerulonephritis, and 5 out of 13 patients were diagnosed with IgA nephropathy (4 new-onset and 1 relapse)[6]. Among them, the partial nephrectomy sample of one patient before vaccination indicated IgA deposition. Therefore, IgA nephropathy after COVID-19 vaccination may be the result of a flare. Moreover, the most common symptom of IgA nephropathy after a COVID-19 vaccination was gross hematuria, and most patients showed a self-limited clinical course without immunosuppression. In addition, eight cases of relapse IgA nephropathy were recorded, and all of them spontaneously recovered within 2 weeks[6].

Thus, the report of this study has shown that the population studied did not develop any form of acute kidney injury after vaccination within the period of study, an indication that COVID-19 is safe.

In addition, the result of normal thyroid function presented in this study is contrary to earlier findings of subacute thyroiditis (SAT) following SARS-CoV-2 vaccination in two out of ten individuals[8]. It was concluded that the autoimmune endocrine disorder after vaccination may develop as a result of either molecular mimicry or the use of adjuvants in vaccine excipients[8]. The mechanism by which SARS-CoV-2 promote SAT, is by reacting with cellular antigens located on thyroid. The spike protein of SAR-CoV-2 may also react with thyroid peroxidase (TPO) antibodies[9].

Form the study above, it was discovered that those with worsening symptoms of SAT, had subacute thyroiditis prior to COVID-19 vaccination. Noting that this type of autoimmune /

inflammatory reactions happens in people genetically predispose to thyroid conditions [9]. Hence, the thyroid dysfunction seen in the above study was concluded that it may be due to genetic factors rather than the COVID-19 vaccine received. It is important to emphasize that, till first quarter of 2022, a total of 10,704,043,684 vaccine doses have been administered[24]. Considering this high number of vaccine doses given worldwide, it was found that the number of published cases about thyroid side-effects is very small (99 cases) [24]. A similar result was found after performing a systematic review of the literature regarding exclusively SAT (and no other thyroid disease) after SARS-CoV-2 vaccination and found 51 patients who developed SAT[4]. Hence, it can be concluded that the incidence of thyroid inconveniences with SARS-CoV-2 vaccination is very rare and negligible.

Nevertheless, an underestimation of these data must be considered, as usual of any scientific research. In fact, this incidence may be higher than that found in the online medicine databases since most physicians do not report (all) cases, many cases go untreated, not everyone gets access to medical/endocrine clinic, there are also ignored cases, etc. All in all, the paucity of published cases should prompt us to conclude that the benefits of SARS-CoV-2 vaccination overweigh the thyroid adverse effects.

To explain the association between SARS-CoV-2 vaccination and thyroid disease, three main mechanisms have been proposed. The first one is shared by different types of vaccines against COVID-19 regardless of the presence of adjuvants in the excipients and is based on molecular mimicry between COVID-19 viral proteins and human tissues. The immune reaction to SARS-CoV-2 Spike Protein and SARS-CoV-2 Nucleo protein leads to the production of cross-reactive antibodies and their interaction with different tissue antigens, including thyroid tissue, may be associated with autoimmune disorders[22]. The second mechanism that could be involved is bystander activation. It is an antigen non-specific

mechanism in which an infection or a vaccination causes a stimulation of innate immunity and finally leads to the activation of autoreactive T cells[26]. Indeed, bystander activation is one of possible pathogenetic mechanisms evoked in autoimmune thyroiditis and Graves' disease[1].

The third mechanism, one of the most frequently repeated postulations in the discussion of different case reports, is linked to the use of adjuvants in vaccine excipients. Adjuvants such as aluminum-based salts, Toll-like receptor (TLR) agonists, emulsions, and other novel adjuvants are critical components of vaccines. They have distinctive physicochemical properties, which can be significant in regulating the strength, duration, and types of immune responses. Furthermore, a hypothetical autoimmune disorder called Autoimmune/autoinflammatory Syndrome Induced by Adjuvants (ASIA) has been proposed to explain autoimmune disorders after vaccination[8]. However, thyroid disease, as other autoimmune diseases, have a complex multifactorial etiology and many factors can contribute to their onset. The exact pathogenetic mechanism that explains the causal link between thyroid disease and vaccination is not yet fully understood and they are also difficult to study, so further data are needed to establish this association with certitude[12].

In addition, a single-center study of patients infected with SARS-CoV-2 who developed thyrotoxicosis were found to have elevated level of inflammatory marker, interleukin-6[7].This study indicated that COVID-19 may be associated with a high risk of thyrotoxicosis, in relationship with systemic immune activation and cytokine storm induced by the SARS-CoV-2 infection. A previous report has also shown interferon and ribavirin therapy–induced thyrotoxicosis[9] In this study, one patient had a significantly increased interleukin-6 level, suggesting a possible role of cytokines in the mRNA vaccine–induced SAT.

The prevalence of slightly elevated level of serum CRP, FT4 and TSH was 1.2% each (one person for each parameter). This finding is consistent with previous study, wherein slight

increase in TSH and FT4 was observed. Nonetheless, the absolute magnitude of change in thyroid function was small[11]. This could also be explained by the changes in thyroid hormone metabolism in non-thyroidal illness syndrome, in which there could be a transient increase in fT4 that coincides with the fall in fT3 while THS is normal[28]. In addition, mRNA vaccines are the most widely used vaccines and it got approval for use under emergency use authorization by the US Food and Drug Administration, thus it is possible that detailed study of the effect of these vaccines on the thyroid and renal function was insufficient Although it is still considered safer over other vaccine types[20].

Therefore, results from this study has shown that the SARS-CoV-2 vaccines does not affect the functionality of both the thyroid and renal systems

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Chapter Five

Conclusion

5.1 Summary of Findings

COVID-19 has continued to be a public health concern worldwide and to date. Different strategies have been put in place to combat the pandemic even as new strains of the virus keep emerging. One of the strategies is the development of the COVID-19 vaccine. Many effective vaccines have been developed and gone through clinical trials why so many are still under trial, yet to be approved. There are concerns for COVID-19 vaccination in triggering renal dysfunction and thyroid autoimmunity thereby causing thyroid dysfunction. Also, data on the effect of preexisting thyroid autoimmunity on the efficacy of COVID-19 vaccination are limited[1]. As of today, the COVID-19 vaccine is still the only effective means to control this pandemic and must be accepted by quite a number of the population to be able to combat the pandemic.

This study had revealed that Pfizer vaccine was the most widely available and received in the cases under study. Hence, it shows that the mRNA vaccines are more used in our environment as compared with the other available and considered safer over other vaccines.

The serum levels of the inflammatory, renal and thyroid function markers of both cases and controls were within the reference range. The values of these biochemical parameters in cases showed no significant difference when compared with the controls ($p>0.05$). Thus, the report of this study has shown that the population studied did not develop any form of acute kidney injury after vaccination within the period of study, an indication that COVID-19 is safe.

In the same vein, the result of normal thyroid function presented in this study for the vaccinated participants were within the reference values. These thyroid results when compared with the none vaccinated group showed no significant difference, a result that is

contrary to an earlier findings of subacute thyroiditis (SAT) following SARS-CoV-2 vaccination in two out of ten individuals. It can be concluded that the autoimmune endocrine disorder after vaccination may develop as a result of either molecular mimicry or the use of adjuvants in vaccine excipients.Hence, SARS-CoV-2 vaccination does not affect thyroid function.

The prevalence of slightly elevated level of serum CRP, FT4 and TSH was 1.2% each (one person for each parameter). This finding is consistent with previous study, wherein slight increase in TSH and FT4 was observed. Nonetheless, the absolute magnitude of change in thyroid function was small. This could also be explained by the changes in thyroid hormone metabolism in non-thyroidal illness syndrome, in which there could be a transient increase in fT4 that coincides with the fall in fT3 while THS is normal.

5.2 Conclusion

COVID-19 vaccination was associated with a modest increase in thyroid hormones, but did not lead to clinically significant thyroid dysfunction 6 months post vaccination. Although there was a mild increase in FT4 and TSH, but was not of any clinical significance or signal to incident of thyroid dysfunction, hence, this goes to proof that thyroid dysfunction following COVID-19 vaccination is rare.

In addition, this study has shown that there is no renal side effect 6 months post vaccination as different from very few studies that reported between 2-3 individuals with glomerulonephritis. Hence, the results from this study has provided important reassurance to people to proceed with COVID-19 vaccination.

5.3 Recommendations

I. The benefits of COVID-19 vaccinations in terms of terminating the pandemic and/or reducing mortality rates far exceed any risk of infrequent complications such as a transient thyroid or renal malfunction. Therefore, vaccine should be made available for all.

II. Renal and thyroid functions checks should be performed if possible before the commencement of SARS-CoV-2 vaccination.

III. Post renal and thyroid function checks should be done six months to one year after vaccination.

IV. Due to the fact that there is paucity of information on these biomarkers in our environment, further prospective studies on these predictive markers with large sample size will be beneficial in discerning the long term effect of different COVID-19 vaccines on the renal and thyroid functions.

5.4 Contribution to Knowledge

The result of this study has shown that the SARS- CoV-2 vaccines does not have any side effect on either the thyroid or renal function in the population studied in Ibadan. Thus giving a reassurance that there are no harm in being vaccinated with SARS-CoV-2 vaccines. In Nigeria, there is vaccine hesitancy because of many unconfirmed reports of negative effect of SARS-CoV-2 vaccines.

In addition, this study has also shown that checking the renal and thyroid functions pre and post vaccination will help to know individuals who are likely to react to the vaccines and as such Clinicians are on the alert to manage such people.

5.5 Suggestion for Further Studies

It will be of great importance to have a longitudinal study of the post vaccination data i.e 6 and 12 months follow up using a larger population, so that different categories of participants can be enrolled. By so doing, there will be a comprehensive follow up of long term effect of SARS-CoV-2 vaccination.

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Appendices

Questionnaire

Potential Effect of SARS-CoV-2 Vaccination on Renal and Thyroid Functions Individuals in Apparently Healthy Population in Ibadan, Nigeria

Dear Respondent,

Famuyiwa Olufisayi Idowu, a Postgraduate Student of the Department of Biological Sciences, Faculty of Natural and Applied Sciences, Lead City University, Ibadan, is Conducting A Study on thePotential Effect of SARS-CoV-2 Vaccination on Renal and Thyroid Functions Individuals in Apparently Healthy Population in Ibadan, Nigeria

a. I Will Appreciate Your Participation in This Study Which Will Involve Us Asking You A Few Questions Regarding Your Experience With SARS-CoV-2 Vaccination and your General Well Being.
b. The Survey Will Take About 5 Minutes to Complete And Whatever Information You Provide Will Be Kept Strictly Confidential. The Study Will Not Pose Any Threat To You And You Are Free To Withdraw From Participating At Any Time.

Thank You for your Time.

If You Agree To Participate In This Study Please Tick This Box [ ]

The information provided will be kept confidential and please do not write your name on it.

Kindly spare a few minutes of your valuable time to answer the questions.

SECTION A: SOCIO-DEMOGRAPHIC CHARACTERISTICS OF RESPONDENTS

1. Age As At Last Birthday; …… Years

2. Sex: Male [ ] Female [ ]

3. Marital Status: A. Single [ ] B. Married [ ] C. Divorced [ ] D. Widowed [ ] E. Separated [ ] F. Others (Please Specify).

4. Occupation:

5. Educational Attainment: A. No Formal Education [ ] B. Primary Education [ ] C. Secondary Education [ ] D. Tertiary Education [ ]

6. Place of residence_____

SECTION B: ANTHROPOMETRIC MEASUREMENT

1. Weight_____
2. Height_____
3. Body mass Index (BMI)_____

4. Blood Presure_____

SECTION C: LIFE STYLE (HISTORY)

1. Cigarette smoking? Before (1) Presently (2) Never (3)
2. If Presently, how? Daily (1) Weekly (2) Occasionally (3)
3. Alcohol intake? Daily (1) Weekly (2) Occasionally (3) Never (4)
4. Vegetables and fruit intake? Daily (1) weekly (2) occasionally (3

SECTION D: MEDICAL HISTORY

1. Do you have any medical condition being managed?

If Yes, Kindly state it_____, and when did it start _____?

2. What medication are you presently on? _____

Have you had any of the following symptoms in the past eight months?

- Cough a) Yes____ b) No_____
- Sore Throat a) Yes____ b) No_____
- Fever a) Yes____ b) No_____
- Fatigue/Tiredness a) Yes____ b) No_____
- Loss of taste or smell a) Yes____ b) No_____
- Diarrhea a) Yes____ b) No_____
- Aches and Pains a) Yes____ b) No_____
- Rash on skin or discoloration of fingers or toes a) Yes____ b) No_____
- Difficulty breathing or shortness of breath a) Yes____ b) No_____
- Chest Pain a) Yes____ b) No_____
- Loss of speech or mobility or confusion a) Yes____ b) No_____

- Have you ever been diagnosed of acute kidney disease/injury? a) Yes____ b) No_____

SECTION E. TEST HISTORY.

- Did you ever test for COVID-19 in the past eight months? a) Yes____ b) No___
- Where were you tested? _____
- Do you have an idea of the method of assay? a) Rapid Testing_____ b) PCR _____
- What was the result a) Positive____ b) Negative_____ c) Indeterminate

SECTION F: VACCINATION HISTORY.

- Have you received the COVID-19 Vaccine? a) Yes____ b) No_____
- If yes, when were you vaccinated? _____
- Are you fully vaccinated _____
- Did you receive any booster dose of the vaccine after fully vaccinated_____
- What type of Covid Vaccine did you receive_____

Did you feel any of the symptoms below after vaccination?

- Cough a) Yes____ b) No_____
- Sore Throat a) Yes____ b) No_____
- Fever a) Yes____ b) No_____
- Fatigue/Tiredness a) Yes____ b) No_____
- Loss of taste or smell a) Yes____ b) No_____
- Diarrhea a) Yes____ b) No_____
- Aches and Pains a) Yes____ b) No_____
- Rash on skin or discoloration of fingers or toes a) Yes____ b) No_____

- Difficulty breathing or shortness of breath a) Yes____ b) No_____
- Chest Pain a) Yes____ b) No_____
- Loss of speech or mobility or confusion a) Yes____ b) No___

Distribution of Participants			
Total Vaccinated before Screening	139	Total Vaccinated after Screening	82
Total Unvaccinated before Screening	145	Total Unvaccinated after Screening	86
Screening Parameters for Both Vaccinated (cases) and Unvaccinated (Control)			
Vaccinated (Total excluded)	57	**Unvaccinated (Total excluded)**	59
HBA1C above 6.5%	14 (24.56%)	HBA1C above 6.5%	6 (10.17%)
Blood Pressure outside the normal range (90/60 - <130/80mmHg)	36 (63.16%)	Blood Pressure outside the normal range (90/60 - <130/80mmHg)	27 (45.76%)
Creatinine above 1.5mg/dl	2 (3.51%)	Creatinine above 1.5mg/dl	3 (5.08%)
Urea above 45mg/dl	2 (3.51%)	Urea above 45mg/dl	5 (8.47%)
Sars-CoV-2 antibody Negative	3 (5.26%)	Sars-CoV-2 antibody Positive	18 (30.51%)

Raw Data from Laboratory Analysis

Unvaccinated

	CRP	CysC	FT3	FT4	TSH	Urea	Creat	Na	K	Cl	HCO_3
1.	1.2	0.5	3.7	11.6	1.3	22.0	0.9	141.0	3.8	102.0	23.0
2.	0.2	0.6	3.7	12.8	1.4	12.0	0.4	140.0	3.9	100.0	22.0
3.	1.1	0.5	4.6	17.4	1.9	15.0	0.5	138.0	4.4	102.0	24.0
4.	1.4	0.5	3.8	14.0	0.7	21.0	0.8	142.0	3.9	104.0	25.0
5.	0.2	0.7	3.3	12.0	0.9	30.0	0.8	137.0	4.0	99.0	26.0
6.	6.9	0.5	3.7	13.7	0.8	16.0	0.8	140.0	3.8	101.0	21.0
7.	0.2	1.1	3.7	19.2	1.41	15.0	0.5	141.0	4.2	104.0	23.0
8.	0.2	0.5	4.6	12.0	1.8	15.0	0.5	136.0	3.9	95.0	27.0
9.	0.5	0.6	3.8	13.6	1.4	21.0	0.7	139.0	3.6	104.0	21.0
10.	1.5	0.8	3.3	16.8	1.3	14.0	0.7	140.0	3.9	102.0	24.0
11.	0.4	0.5	3.7	18.0	1.4	10.0	0.5	142.0	4.2	105.0	23.0
12.	0.2	0.8	3.7	14.3	1.9	18.0	0.7	141.0	3.9	103.0	24.0
13.	0.5	1.0	4.6	12.9	0.7	21.0	0.8	136.0	4.2	97.0	21.0
14.	0.4	0.7	3.8	11.7	0.9	18.0	0.9	137.0	4.1	99.0	24.0

15. 0.2	0.9	3.3	10.9	0.8	23.0	0.9	141.0	3.6	103.0	24.0
16. 0.5	1.0	3.42	12.9	1.41	12.0	0.5	139.0	3.5	99.0	26.0
17. 0.6	0.8	4.61	13.9	1.8	14.0	0.5	136.0	4.1	98.0	24.0
18. 1.3	0.9	3.51	14.6	1.4	18.0	1.0	138.0	4.0	101.0	23.0
19. 1.3	0.4	3.0	14.8	0.895	21.0	1.0	140.0	4.2	102.0	24.0
20. 0.4	0.7	4.1	11.5	2.21	17.0	1.2	144.0	3.6	105.0	25.0
21. 0.9	0.5	3.64	15.8	3.32	28.0	1.1	141.0	4.1	106.0	21.0
22. 0.3	0.7	4.59	17.9	0.993	42.0	0.7	140.0	3.8	100.0	26.0
23. 0.6	0.8	5.22	12.1	3.51	23.0	0.8	139.0	4.4	100.0	25.0
24. 2.0	1.0	4.36	12.9	2.222	21.0	0.9	136.0	4.1	98.0	24.0
25. 3.3	0.9	4.52	14.3	0.876	19.0	0.9	138.0	4.0	101.0	23.0
26. 4.1	1.0	4.58	17.9	0.543	40.0	0.5	140.0	4.2	102.0	24.0
27. 1.1	0.8	3.78	20.0	1.123	25.0	0.5	144.0	3.6	105.0	25.0
28. 1.1	0.9	3.99	19.3	1.78	17.0	0.8	139.0	4.9	104.0	22.0
29. 0.2	0.8	4.25	16.4	0.7	18.0	0.7	137.0	4.3	96.0	27.0
30. 0.2	0.7	3.7	13.8	0.9	26.0	1.0	136.0	3.8	96.0	26.0
31. 0.2	0.7	4.6	12.5	0.8	14.0	0.9	137.0	4.2	96.0	27.0
32. 0.2	0.5	3.8	16.9	1.41	29.0	1.0	141.0	3.6	102.0	25.0
33. 0.5	1.1	3.3	12.7	1.8	26.0	1.0	137.0	4.0	102.0	21.0
34. 1.5	0.5	3.42	15.3	1.4	17.0	0.6	142.0	4.1	102.0	36.0
35. 0.4	0.8	4.61	12.7	0.895	23.0	0.9	139.0	3.8	104.0	21.0
36. 0.2	0.8	3.51	13.6	2.21	22.0	1.0	141.0	3.9	107.0	20.0
37. 0.5	0.5	3.0	17.4	3.32	22.0	1.0	137.0	4.2	96.0	27.0
38. 0.4	0.8	4.1	12.8	0.993	12.0	0.8	141.0	3.6	102.0	25.0
39. 0.2	1.0	3.64	11.9	3.51	15.0	0.9	137.0	4.0	102.0	21.0

40.	0.5	0.7	4.59	10.9	2.222	21.0	1.0	142.0	4.1	102.0	36.0
41.	0.6	0.9	5.22	12.0	0.876	30.0	0.9	139.0	3.8	104.0	21.0
42.	1.3	1.0	4.36	14.2	0.543	16.0	0.9	141.0	3.9	107.0	20.0
43.	1.3	0.8	4.52	16.8	1.123	15.0	1.0	137.0	4.2	96.0	27.0
44.	0.4	0.9	4.58	17.4	1.78	15.0	1.1	141.0	3.6	102.0	25.0
45.	0.9	0.4	3.78	12.7	0.8	21.0	0.5	137.0	4.0	102.0	21.0
46.	0.3	0.6	3.99	15.3	1.41	14.0	1.1	142.0	4.1	102.0	36.0
47.	0.6	0.5	4.25	12.7	1.8	10.0	0.9	139.0	3.8	104.0	21.0
48.	0.2	0.8	3.7	13.6	1.4	18.0	0.8	141.0	3.9	107.0	20.0
49.	0.5	0.9	3.7	17.4	0.895	21.0	0.9	140.0	3.9	100.0	22.0
50.	0.4	0.4	4.6	12.8	2.21	18.0	1.0	138.0	4.4	102.0	24.0
51.	0.2	0.7	3.8	11.9	3.32	23.0	0.9	142.0	3.9	104.0	25.0
52.	0.5	0.5	3.3	10.9	2.1	12.0	0.9	137.0	4.0	99.0	26.0
53.	0.6	0.7	3.7	12.0	3.21	14.0	1.0	140.0	3.8	101.0	21.0
54.	0.8	0.8	3.7	14.2	1.98	18.0	1.1	141.0	4.2	104.0	23.0
55.	0.6	1.0	4.6	16.8	0.84	21.0	1.3	136.0	3.9	95.0	27.0
56.	0.2	0.9	3.8	17.4	4.0	17.0	1.1	139.0	3.6	104.0	21.0
57.	0.5	1.0	3.3	12.8	2.0	28.0	0.9	140.0	3.9	102.0	24.0
58.	0.4	0.8	3.7	17.4	0.576	42.0	0.8	142.0	4.2	105.0	23.0
59.	0.2	0.9	3.7	14.0	0.491	22.0	0.8	141.0	3.9	103.0	24.0
60.	0.5	0.8	4.6	12.0	1.87	12.0	0.9	140.0	3.9	100.0	22.0
61.	0.0	0.7	3.8	13.7	0.895	15.0	0.9	138.0	4.4	102.0	24.0
62.	0.6	0.7	3.51	19.2	2.21	21.0	0.5	142.0	3.9	104.0	25.0
63.	0.2	0.5	3.0	12.0	3.32	30.0	0.5	137.0	4.0	99.0	26.0
64.	0.5	1.1	4.1	13.6	2.1	16.0	0.8	140.0	3.8	101.0	21.0

65. 0.4	0.5	3.64	16.8	3.21	15.0	0.7	141.0	4.2	104.0	23.0
66. 0.2	0.8	4.59	18.0	1.98	15.0	1.0	136.0	3.9	95.0	27.0
67. 0.5	0.8	5.22	14.3	0.84	21.0	0.9	139.0	3.6	104.0	21.0
68. 0.3	0.5	4.36	12.9	4.0	14.0	1.0	140.0	3.9	102.0	24.0
69. 1.1	1.1	4.52	11.7	2.0	10.0	1.0	142.0	4.2	105.0	23.0
70. 2.5	1.0	4.58	12.4	0.576	18.0	1.1	141.0	3.9	103.0	24.0
71. 0.2	0.7	3.78	13.3	0.491	21.0	0.9	140.0	3.9	100.0	22.0
72. 0.2	0.8	3.99	13.9	1.87	18.0	0.8	138.0	4.4	102.0	24.0
73. 0.2	1.0	4.25	18.5	0.942	23.0	0.9	142.0	3.9	104.0	25.0
74. 0.5	0.7	3.5	16.2	1.89	12.0	0.9	137.0	4.0	99.0	26.0
75. 1.5	0.9	4.0	18.8	2.51	14.0	0.5	140.0	3.8	101.0	21.0
76. 0.4	0.4	3.61	11.9	0.491	18.0	0.5	141.0	4.2	104.0	23.0
77. 0.2	0.6	3.22	19.0	1.87	21.0	0.8	136.0	3.9	95.0	27.0
78. 0.5	1.1	4.7	14.7	0.942	17.0	0.7	139.0	3.6	104.0	21.0
79. 0.4	0.7	3.9	18.0	1.89	28.0	1.0	140.0	3.9	102.0	24.0
80. 0.2	0.9	3.5	11.6	2.51	22.0	0.9	142.0	4.2	105.0	23.0
81. 0.5	0.8	3.12	12.8	4.0	19.0	1.0	141.0	3.9	103.0	24.0
82. 0.6	0.7	4.1	17.4	2.0	25.0	1.0	140.0	3.9	100.0	22.0
83. 3.2	0.7	3.85	14.0	0.576	29.0	1.2	138.0	4.4	102.0	24.0
84. 0.7	0.5	3.5	12.0	0.491	30.0	0.9	142.0	3.9	104.0	25.0
85. 0.6	1.1	4.0	13.7	1.87	19.0	0.9	137.0	4.0	99.0	26.0
86. 3.2	0.8	3.61	19.2	2.04	22.0	0.6	140.0	3.8	101.0	21.0

Source: Author's Field Work.

Raw Data from Laboratory Analysis

Vaccinated

	CRP	CysC	FT3	FT4	TSH	Urea	Creat	Na	K	Cl	HCO_3
1.	0.9	0.7	3.6	13.7	0.9	26.0	1.1	141.0	4.2	104.0	23.0
2.	0.4	0.5	3.4	12.9	0.9	10.0	0.5	136.0	3.9	95.0	27.0
3.	0.6	0.8	4.4	13.1	1.1	27.0	0.8	139.0	3.6	104.0	21.0
4.	2.9	0.8	3.9	10.9	0.8	14.0	0.7	140.0	3.9	102.0	24.0
5.	0.5	0.8	3.6	12.5	0.9	29.0	1.0	142.0	4.2	105.0	23.0
6.	0.2	0.7	3.4	16.9	1.1	10.0	0.5	141.0	3.9	103.0	24.0
7.	8.1	0.5	4.4	20.2	0.8	27.0	0.8	140.0	3.9	100.0	22.0
8.	0.4	0.8	3.9	20.1	0.9	14.0	0.7	138.0	4.4	102.0	24.0
9.	0.6	0.8	3.6	14.1	1.1	29.0	1.0	142.0	3.9	104.0	25.0
10.	0.2	0.8	3.4	12.9	0.8	15.0	0.6	131.0	3.8	91.0	26.0
11.	0.9	0.7	4.4	12.3	1.0	18.0	0.6	141.0	3.9	103.0	24.0
12.	0.4	0.8	3.9	11.1	1.3	17.0	0.7	136.0	3.9	99.0	23.0
13.	0.6	0.8	3.6	11.3	1.4	28.0	0.9	139.0	4.1	102.0	23.0
14.	0.2	0.5	3.4	13.9	1.9	26.0	0.9	142.0	4.3	105.0	23.0

15.	0.8	0.6	4.4	18.5	0.7	24.0	1.1	137.0	3.9	97.0	26.0
16.	1.1	0.9	3.9	16.2	0.9	22.0	1.0	135.0	3.3	96.0	24.0
17.	1.5	0.4	4.0	18.8	0.8	26.0	0.4	140.0	4.1	100.0	26.0
18.	0.2	0.6	4.58	11.9	1.41	16.0	0.6	140.0	4.0	101.0	25.0
19.	0.7	0.8	3.6	19.0	1.8	15.0	0.7	140.0	3.9	99.0	27.0
20.	0.5	0.9	3.99	14.7	1.4	18.0	1.1	140.0	4.1	104.0	22.0
21.	0.9	0.8	4.25	18.0	0.895	14.0	0.4	139.0	4.1	102.0	23.0
22.	2.1	0.7	3.6	11.6	2.21	10.0	0.6	142.0	4.3	105.0	23.0
23.	0.9	0.9	3.7	12.9	3.32	15.0	0.7	137.0	3.9	97.0	26.0
24.	0.2	0.6	4.6	14.4	0.993	20.0	1.1	140.0	3.8	101.0	25.0
25.	0.5	0.9	3.8	17.5	3.51	19.0	1.0	140.0	3.9	102.0	24.0
26.	1.5	0.7	3.3	12.9	0.993	15.0	0.5	141.0	3.9	103.0	24.0
27.	0.2	0.5	3.9	12.8	3.51	18.0	0.8	140.0	3.9	100.0	22.0
28.	0.7	0.8	3.7	11.9	2.222	14.0	0.7	138.0	4.4	102.0	24.0
29.	0.5	0.8	4.2	13.9	0.876	10.0	1.0	142.0	3.9	104.0	25.0
30.	0.9	0.8	3.8	21.3	0.543	15.0	0.5	131.0	3.8	91.0	26.0
31.	2.1	0.7	3.3	20.2	1.123	20.0	0.8	141.0	3.9	103.0	24.0
32.	3.5	0.5	3.8	17.5	1.78	27.0	0.7	136.0	3.9	99.0	23.0
33.	0.6	0.8	3.3	15.2	0.8	14.0	1.0	139.0	4.1	102.0	23.0
34.	0.7	0.8	3.9	16.0	1.54	29.0	0.6	142.0	4.3	105.0	23.0
35.	0.5	0.8	3.7	17.0	1.123	10.0	0.6	137.0	3.9	97.0	26.0
36.	2.1	0.7	4.2	19.9	1.4	27.0	0.7	135.0	3.3	96.0	24.0
37.	0.9	0.8	3.8	11.7	0.999	14.0	1.3	140.0	3.9	100.0	22.0
38.	0.4	0.8	3.3	16.6	0.743	29.0	0.9	138.0	4.4	102.0	24.0
39.	0.6	0.5	3.8	16.2	1.89	15.0	1.1	142.0	3.9	104.0	25.0

40. 2.9	0.6	3.3	17.9	2.51	18.0	1.0	137.0	4.0	99.0	26.0
41. 0.5	0.9	3.41	14.6	4.0	17.0	0.4	140.0	3.8	101.0	21.0
42. 0.2	0.4	4.61	18.9	2.22	28.0	0.6	141.0	4.2	104.0	23.0
43. 0.9	0.6	4.0	11.4	0.576	26.0	0.7	136.0	3.9	95.0	27.0
44. 0.4	0.8	3.0	15.5	0.491	24.0	1.1	139.0	3.6	104.0	21.0
45. 0.6	0.9	4.1	11.2	1.777	22.0	0.4	140.0	3.9	102.0	24.0
46. 0.2	0.8	3.64	14.4	2.11	26.0	0.6	142.0	4.2	105.0	23.0
47. 0.1	0.7	4.59	18.0	1.123	16.0	0.7	141.0	3.9	103.0	24.0
48. 0.4	0.9	4.2	15.7	1.78	15.0	1.1	140.0	3.9	100.0	22.0
49. 0.8	0.6	3.8	14.0	0.8	27.0	1.0	138.0	4.4	102.0	24.0
50. 1.5	0.9	3.3	12.0	1.54	14.0	0.7	142.0	3.9	104.0	25.0
51. 1.3	1.0	3.8	13.7	1.123	29.0	1.0	137.0	4.0	99.0	26.0
52. 0.5	0.7	3.3	19.2	1.42	10.0	0.6	144.0	4.0	109.0	21.0
53. 0.3	1.1	3.8	13.7	0.999	27.0	0.6	142.0	3.9	106.0	22.0
54. 3.2	1.1	3.7	12.9	0.743	14.0	0.7	139.0	3.7	104.0	21.0
55. 1.9	0.9	3.9	13.1	1.89	29.0	1.3	139.0	3.6	98.0	27.0
56. 0.9	0.8	3.8	10.9	2.51	15.0	0.9	137.0	4.0	97.0	26.0
57. 2.3	0.7	3.3	12.5	3.992	18.0	1.1	138.0	3.6	99.0	24.0
58. 3.2	0.7	3.8	16.9	2.22	17.0	1.0	140.0	3.9	103.0	23.0
59. 0.4	0.5	3.3	15.8	0.576	28.0	0.4	140.0	3.9	100.0	22.0
60. 1.5	0.7	3.41	15.9	1.15	26.0	0.6	138.0	4.4	102.0	24.0
61. 0.2	0.8	4.0	21.0	1.822	24.0	0.7	142.0	3.9	104.0	25.0
62. 0.7	0.6	4.0	13.0	2.11	22.0	1.1	137.0	4.0	99.0	26.0
63. 0.5	1.0	3.42	13.2	2.23	26.0	0.4	144.0	4.0	109.0	21.0
64. 0.9	0.7	3.91	13.8	0.987	16.0	0.6	142.0	3.9	106.0	22.0

65. 2.1	1.1	3.45	12.0	0.89	15.0	0.7	139.0	3.7	104.0	21.0
66. 0.9	1.1	3.98	19.9	4.41	17.0	1.1	141.0	3.9	103.0	24.0
67. 0.6	0.9	3.41	20.1	0.589	19.0	1.0	140.0	3.9	100.0	22.0
68. 0.7	0.8	4.0	12.5	0.654	22.0	1.2	138.0	4.4	102.0	24.0
69. 0.5	0.7	4.0	16.9	1.1	23.0	0.9	142.0	3.9	104.0	25.0
70. 1.5	0.7	3.42	15.8	2.34	20.0	0.8	131.0	3.8	91.0	26.0
71. 0.2	0.5	3.91	15.9	3.992	22.0	0.6	141.0	3.9	103.0	24.0
72. 0.7	0.7	3.45	21.0	2.22	26.0	0.5	136.0	3.9	99.0	23.0
73. 0.5	0.8	3.98	13.0	0.576	16.0	0.5	139.0	4.1	102.0	23.0
74. 0.9	0.6	4.22	12.0	0.654	15.0	0.8	142.0	4.3	105.0	23.0
75. 2.1	0.7	5.0	18.1	0.742	17.0	0.7	137.0	3.9	97.0	26.0
76. 3.5	0.7	4.1	15.8	0.642	19.0	1.1	135.0	3.3	96.0	24.0
77. 0.6	0.5	3.91	16.1	3.992	22.0	1.0	140.0	4.1	100.0	26.0
78. 0.7	0.7	4.4	12.2	2.22	23.0	1.2	140.0	4.0	101.0	25.0
79. 0.5	0.8	5.0	13.6	0.576	20.0	0.6	140.0	3.9	99.0	27.0
80. 0.6	0.6	4.0	12.4	0.654	21.0	0.5	140.0	4.1	104.0	22.0
81. 0.9	0.9	3.42	15.0	3.992	19.0	0.5	139.0	4.1	102.0	23.0
82. 1.1	0.6	3.91	11.5	2.22	12.0	0.8	140.0	4.1	104.0	22.0

Source: Author's Field Work.

Unvaccinated

	Weight	Height	SystP	DiastP	HbA1C
1.	92.0	189.0	117.0	69.0	5.4
2.	74.0	170.0	121.0	81.0	5.2
3.	69.0	162.0	119.0	81.0	5.4
4.	64.0	175.0	129.0	78.0	5.9
5.	84.0	176.0	121.0	74.0	5.5
6.	70.0	167.0	121.0	85.0	5.0
7.	60.0	170.0	127.0	70.0	5.1
8.	57.0	167.0	109.0	60.0	5.9
9.	82.0	167.0	120.0	88.0	6.1
10.	49.0	162.0	113.0	73.0	5.8
11.	63.0	155.0	125.0	68.0	6.3
12.	66.0	158.0	117.0	78.0	5.2
13.	70.0	180.0	120.0	79.0	5.4
14.	67.0	165.0	123.0	73.0	6.0
15.	65.0	167.0	138.0	82.0	5.5

16. 85.0 177.0 128.0 66.0 6.0

17. 90.0 177.0 138.0 95.0 4.5

18. 59.0 170.0 127.0 94.0 6.1

19. 80.0 164.0 140.0 80.0 5.4

20. 76.0 170.0 114.0 60.0 4.6

21. 77.0 170.0 120.0 98.0 4.9

22. 74.0 178.0 116.0 82.0 5.7

23. 68.0 183.0 114.0 73.0 5.3

24. 60.0 165.0 120.0 86.0 5.1

25. 71.0 160.0 116.0 73.0 5.3

26. 73.0 170.0 138.0 75.0 5.6

27. 58.0 171.0 120.0 80.0 5.3

28. 81.0 170.0 126.0 83.0 4.8

29. 64.0 154.0 121.0 86.0 4.9

30. 64.0 164.0 122.0 69.0 5.3

31. 57.0 177.0 128.0 73.0 4.8

32. 79.0 169.0 118.0 78.0 5.3

33. 69.0 172.0 123.0 93.0 5.3

34. 61.0 178.0 126.0 84.0 5.0

35. 62.0 161.0 121.0 67.0 5.2

36. 58.0 164.0 122.0 73.0 5.7

37. 65.0 175.0 128.0 92.0 4.5

38. 69.0 173.0 118.0 69.0 4.6

39. 70.0 169.0 123.0 73.0 5.0

40. 60.0 182.0 127.0 78.0 5.2

41.	63.0	165.0	109.0	93.0	5.0
42.	80.0	172.0	120.0	84.0	6.4
43.	85.0	177.0	113.0	67.0	3.6
44.	72.0	173.0	125.0	73.0	4.6
45.	70.0	173.0	117.0	92.0	5.2
46.	66.0	170.0	120.0	76.0	5.2
47.	66.0	165.0	123.0	93.0	5.0
48.	78.0	161.0	138.0	94.0	6.0
49.	80.0	163.0	128.0	67.0	5.2
50.	80.0	177.0	138.0	79.0	5.4
51.	70.0	183.0	127.0	79.0	5.4
52.	65.0	170.0	140.0	91.0	5.2
53.	80.0	168.0	114.0	65.0	5.3
54.	59.0	157.0	123.0	70.0	5.6
55.	60.0	171.0	127.0	71.0	4.9
56.	65.0	171.0	134.0	91.0	5.2
57.	76.0	160.0	121.0	81.0	5.3
58.	68.0	171.0	131.0	81.0	4.2
59.	69.0	170.0	120.0	86.0	5.2
60.	62.0	169.0	133.0	87.0	4.9
61.	53.0	164.0	130.0	80.0	5.0
62.	66.0	172.0	133.0	91.0	5.2
63.	64.0	162.0	111.0	86.0	4.7
64.	61.0	168.0	116.0	72.0	5.0
65.	86.0	164.0	144.0	82.0	5.0

66.	54.0	171.0	125.0	97.0	4.5
67.	59.0	168.0	130.0	91.0	4.4
68.	62.0	167.0	140.0	79.0	4.8
69.	72.0	179.0	138.0	80.0	5.0
70.	57.0	161.0	125.0	79.0	5.3
71.	65.0	167.0	120.0	97.0	5.0
72.	51.0	161.0	120.0	83.0	5.6
73.	73.0	166.0	123.0	77.0	4.6
74.	80.0	168.0	122.0	75.0	4.7
75.	61.0	172.0	121.0	79.0	4.5
76.	74.0	159.0	119.0	63.0	5.5
77.	65.0	169.0	141.0	92.0	4.8
78.	52.0	158.0	125.0	85.0	5.6
79.	86.0	163.0	137.0	84.0	5.0
80.	91.0	165.0	127.0	99.0	5.6
81.	76.0	164.0	120.0	80.0	5.3
82.	59.0	172.0	131.0	91.0	5.0
83.	59.0	170.0	131.0	86.0	5.0
84.	60.0	175.0	122.0	72.0	5.2
85.	70.0	177.0	131.0	82.0	5.5
86.	70.0	186.0	120.0	97.0	5.5

Source: Author,s Field Work

Deleted[Azuka Patrick]: ”

Vaccnated

	Weight	Height	SystP	DiastP	HbA1C
	Kg	**cm**	**mm/hg**		**mmol/mol**
1.	61.0	176.0	126.0	91.0	4.6
2.	70.0	181.0	121.0	79.0	5.5
3.	75.0	169.0	122.0	80.0	5.1
4.	53.0	164.0	117.0	79.0	4.9
5.	63.0	170.0	142.0	97.0	4.4
6.	68.0	170.0	123.0	83.0	4.6
7.	93.0	184.0	122.0	77.0	5.7
8.	74.0	172.0	121.0	75.0	5.7
9.	68.0	176.0	119.0	79.0	4.8
10.	75.0	169.0	118.0	63.0	5.8
11.	72.0	180.0	117.0	92.0	5.9
12.	64.0	168.0	142.0	85.0	5.7
13.	76.0	178.0	123.0	84.0	5.3
14.	95.0	180.0	122.0	99.0	4.7

15.	65.0	167.0	121.0	80.0	5.5
16.	67.0	174.0	119.0	87.0	5.7
17.	50.0	164.0	121.0	80.0	4.4
18.	70.0	171.0	127.0	82.0	4.5
19.	74.0	173.0	109.0	94.0	5.7
20.	78.0	172.0	120.0	68.0	5.3
21.	68.0	162.0	113.0	78.0	5.0
22.	88.0	177.0	125.0	80.0	5.7
23.	60.0	161.0	117.0	87.0	5.0
24.	83.0	175.0	120.0	81.0	5.5
25.	70.0	174.0	123.0	98.0	4.9
26.	65.0	169.0	123.0	90.0	5.1
27.	65.0	175.0	138.0	78.0	5.3
28.	55.0	165.0	128.0	80.0	5.0
29.	74.0	176.0	138.0	76.0	4.6
30.	76.0	172.0	127.0	75.0	5.0
31.	59.0	165.0	140.0	93.0	4.8
32.	60.0	164.0	114.0	80.0	4.3
33.	63.0	163.0	123.0	74.0	4.6
34.	54.0	162.0	127.0	81.0	4.8
35.	66.0	177.0	134.0	93.0	5.6
36.	58.0	169.0	121.0	81.0	4.7
37.	64.0	165.0	131.0	87.0	4.3
38.	73.0	178.0	120.0	79.0	4.5
39.	59.0	151.0	127.0	73.0	5.0

40.	67.0	173.0	134.0	93.0	5.0
41.	62.0	158.0	121.0	72.0	4.6
42.	54.0	162.0	131.0	91.0	5.0
43.	79.0	160.0	120.0	82.0	4.5
44.	83.0	162.0	127.0	82.0	5.0
45.	53.0	170.0	134.0	79.0	5.0
46.	59.0	176.0	121.0	91.0	5.3
47.	66.0	187.0	131.0	72.0	5.0
48.	72.0	170.0	120.0	91.0	4.1
49.	69.0	163.0	123.0	73.0	5.0
50.	58.0	168.0	127.0	77.0	5.1
51.	69.0	174.0	134.0	75.0	5.2
52.	73.0	159.0	121.0	80.0	5.7
53.	60.0	174.0	131.0	84.0	4.6
54.	61.0	166.0	120.0	68.0	5.1
55.	72.0	168.0	133.0	73.0	5.3
56.	76.0	180.0	130.0	80.0	5.0
57.	76.0	166.0	133.0	67.0	5.3
58.	77.0	165.0	111.0	65.0	5.0
59.	84.0	193.0	121.0	80.0	4.4
60.	66.0	171.0	119.0	81.0	5.3
61.	60.0	179.0	129.0	81.0	4.5
62.	83.0	171.0	121.0	76.0	5.7
63.	64.0	167.0	121.0	85.0	5.2
64.	81.0	181.0	127.0	81.0	4.8

65.	72.0	178.0	121.0	82.0	5.4
66.	65.0	167.0	131.0	72.0	4.4
67.	69.0	171.0	120.0	79.0	5.0
68.	84.0	168.0	133.0	78.0	4.9
69.	75.0	158.0	130.0	78.0	4.7
70.	59.0	167.0	133.0	87.0	4.8
71.	70.0	172.0	111.0	96.0	5.5
72.	55.0	166.0	121.0	73.0	4.9
73.	84.0	161.0	119.0	76.0	4.8
74.	61.0	165.0	129.0	72.0	4.5
75.	69.0	169.0	121.0	78.0	4.8
76.	68.0	165.0	121.0	63.0	4.8
77.	60.0	165.0	127.0	73.0	5.2
78.	74.0	162.0	119.0	76.0	5.0
79.	75.0	172.0	129.0	72.0	5.4
80.	66.0	175.0	121.0	78.0	5.4
81.	67.0	164.0	121.0	80.0	5.1
82.	56.0	168.0	127.0	77.0	5.5

Source: Author's Field Work, 2023.

INFORMED CONSENT FORM

IRB Research approval number:

This approval will elapse on:

Title of the research: **Potential Effects of SARS-CoV-2 Vaccination on Renal and Thyroid Functions in Apparently Healthy Population in Ibadan, Nigeria**

Name and affiliation of researcher of applicant: Mrs. FAMUYIWA, Olufisayo Idowu, DEPARTMENT OF BIOLOGICAL SCIENCE, FACULTY OF NATURAL AND APPLIED SCIENCES, LEAD CITY UNIVERSITY, IBADAN. NIGERIA

Sponsor of research: The research is self-sponsored.

Purpose of research: Information emanating from this study will help to know the effect of SARS-CoV-2 vaccination on thyroid and renal function

Procedure of the research, what shall be required of each participant and approximate total number of participants that would be involved in the research: 10mLs of blood will be required from each participants.

Costs to the participants of joining the research: This research will be at no cost to the participants.

Benefit: The result from this research will help to know the effect of SARS-CoV-2 vaccination on thyroid and renal functions. It will assist the Clinicians to know if there will be any side effect of SARS- CoV-2 vaccination on thyroid and renal function parameters of individuals that had been vaccinated.

Confidentiality: All information collected in this study will be given code numbers and no name will be recorded and your name or any identifier will not be used in any publication or reports from this study. As part of my responsibility to conduct this research properly, officials from NAFDAC, NHREC and ethics and similar agencies may check for appropriateness of my research processes.

Voluntariness: Your participation in this study is purely voluntary.

Alternative to participation: Your refusal to participate in this study will not affect your work or treatment.

Due inducement: You will not be paid any fees for participating in this research**.**

Consequences of participant's decision to withdraw from research and procedure for orderly termination of participation: You can choose to withdraw from the research at any time. Please note that some of the information that has been obtained about you before you

chose to withdraw may have been modified or used in reports and publications. These cannot be retrieved anymore. However the researchers promise to comply with your wishes as much as is practicable.

Modality of providing treatments and action to be taken in case of injury or adverse events: The chance of an adverse effect is rare but if you suffer any injury as a result of your participation in this research, you will be treated at the University of Ibadan Teaching Hospital and the researcher will bear the cost of this treatment.

What happens to research participants and communities when the research is over: The researcher will inform you of the outcome of the research if any one shows interest. During the course of this research, you will be informed about any information that may affect your continued participation or your health.

Statement of person obtaining informed consent:

I have fully explained this research to ---------- and have given sufficient information, including the risks and benefits, to make an informed decision.

DATE---------- SIGNATURE----------

NAME----------

Statement of person giving consent: I have read the description of the research or have had it translated to the language I understand. I have also talked over with the doctor to my satisfaction. I understand that my participation is voluntary. I know enough about the purpose, methods, risks, and benefits of the research study to judge that I want to take part in it. I understand that I may freely stop being part of this study at any time. I have a copy of this consent form and additional information sheet to keep for myself.

DATE----------

SIGNATURE----------

SUBJECT' CODE----------

WITNESS' SIGNATURE----------

Bio-Data

A. Personal Data:

1. Full Name:	Olufiayo Idowu FAMUYIWA
	18, Falola Street, Idi-Mango, New- Felele Area,
	Soka, Ibadan.
	Fissymike05@yahoo.com
	08033705657
2. Date And Place Of Birth	14th September, 1977, Ibadan
3. Nationality	Nigerian
4. State Of Origin	Ogun
5. Name And Address Of Next Of Kin	Mr. Michael Abayomi Famuyiwa
	18, Falola Street, New Felele Area.

B. Educational Background

Educational Institutions Attended with Dates and Qualification

School Attended	**Date**	**Qualification**

Christ Church School Mapo III	1983-1988	First Leaving Sch. Cert.
Methodist Secondary School	1989- 1991	Junior School Certificate
Wesley College of Science	1991-1994	West African Exam
Scool of Med. Lab. Science	1995-1999	Assoc. Med. Lab Science
University Of Ibadan	2013-2015	M.Sc Chemical Pathology
Lead City University	2019 -2023	Ph.D
National Youth Service Corps	2000-2001	NYSC Certificate

C. Work Experience	**Date**
University College Hospital	2001 – Till date

D. Awards and Fellowships (If any) Best Graduating Student (1999)

E. Membership of Academic Professional Bodies

a. Corresponding Member, Point of Care Testing (TF-POCT), International Federation of Clinical Chemists

b. Member: Association of Clinical Chemist of Nigeria

c. Member, Laboratory Quality Manual Committee for Chemical Pathology Department, UCH

F. Publication

1. Thesis /Dissertation

a. Folliculogenesis in Premenopausal African Blacks with Metabolic Syndrome: Association of Inhibin B with Anthropometry, Lipids, Adipokines, Sex Hormones and their binding Globulin

b. Relationship between Maternal and Infants' Plasma and Breast Milk levels of β- Carotene, Ascorbic acid, Tocopherol, Selenium and Zinc

c. Correlation between body fat distribution and Uric acid Metabolism in College Students

2. Book/Monographs Nil

3. Scholarly articles

1. Famuyiwa, I.O., Bitrus D.P., Charles-Davies, M. A., Fabian, U.A. , Fasanmade, A.A., Olaniyi, J.A., Oyewole, O.E., Owolabi, M.O., Adebusuyi, J.R., Hassan, O, Ajobo, B.M. Ebesunun, M.O., Adigun, K., Akinlade, K.S., Okoli, S.U., Arinola, O.G and Agbedana E.O. (2016). Reproductive function in Premenopausal African Blacks with Metabolic Syndrome: Associations among Inhibin B, Adipokines, Pituitary and Sex Hormones and Sex Hormone Binding Globulin. International Journal of Medicine and Medical Sciences Vol. 8. No. 8: 84-90.

2. Arinola, G.O., Fashina, O.A., Oluyomi Ishola, O.C., Akinbola, O.I., Akinbile, S.A., Eegunjobi, A.O., Bello, M.D., Edem, F.V., Rahamon, S.k., Famuyiwa, O.I., Olaoto,A.J., Olaniyan, O.A., Oke, A.C., Fowotade, A.,Abimbola, O., Johnson, J.O., Fagbemi, O.S., Salami, F., and Alonge T.O. (2021). Demographic attributes of COVID-19 patients in an Infectious Disease Center of Nigeria. African Journal of Clinical and Experimental Microbiology. Vol. 22 (1): 21-27

3. Kotila, T.R., Alonge, T.O., Fowotade, A., Famuyiwa, O.I. and Akinbile A.S. (2021). Association of the ABO blood group with SARS-CoV-2 infection in a commun.ity with low infection rate. International Society of Blood Transfusion.

4. Notable Scholarly Accomplishments Nil

5. Major Conferences

- 15th National Annual Public Health Lecture Series of Association of Medical Laboratory Scientists of Nigeria- Virtual attendance (2021)
- TAUBER Bioinformatics Research Training on Analysis of SARS-COV-2 Genomic Data, Nigeria (2020)
- Training of Laboratory Quality Management System Mentors. Abuja, Nigeria (2020)
- 56th Annual Medical Laboratory Scientists Conference of Association of Medical Laboratory Scientists of Nigeria, Ebonyi, Nigeria- Virtual attendance (2020)
- Training on Bioinformatics and Molecular Biology. Lagos, Nigeria (2019)
- 55th Annual Medical Laboratory Scientists Conference of Association of Medical Laboratory Scientists of Nigeria, Abuja, Nigeria- Virtual attendance (2019)
- Roche Experience scientific Meeting 2018. Johannesburg, South Africa (2018)
- Conference on Strategic Innovation for Community Health Program. Nairobi, Kenya (2018)
- Training on Leadership and Management for Healthcare Leaders and Managers. GIMPA, Ghana
- Training on Medical Laboratory Inspection. Abuja, Nigeria (2017)
- Roche Diagnostic training on COBAS Ampliprep- COBAS Taqman, UCH. Nigeria (2016)
- Roche Diagnostic training on COBAS 6000 Operator training Basic. Johannesburg, South Africa (October, 2016)

…… ……..

Signature Date

Deleted[Azuka Patrick]:

The University Compliance Certification

This is to certify that the Thesis of **Olufisayo Idowu Famuyiwa** with Matriculation Number **LCU/PG/001434**, in the Department of Biological Science, Faculty of Natural and Applied Sciences, Lead City University, Ibadan, Nigeria is in full compliance with the approved University format and style.

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Signatures Date