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**INFORMATION**

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# A NEONATAL EARLY WARNING SYSTEM (NEWS) IN PORT MORESBY GENERAL HOSPITAL, SPECIAL CARE NURSERY

Running title: Use of Neonatal Early Warning System

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**ABSTRACT**:
The neonatal mortality rate in Papua New Guinea is high, estimated at 24 per 1000 live births. The neonatal case fatality rate in newborns in provincial and referral hospitals was 5.9% in 2021. Deteriorating newborns can be difficult to identify. This observational study investigated the feasibility of using a neonatal colour coded observation and response chart to identify neonates at risk of deterioration and to promptly escalate care in the Special Care Nursery of Port Moresby General Hospital. The chart was adapted from the Plymouth Hospital Neonatal Early Warning System chart, and was used to collect data over 8 weeks between 1st May and 30th June 2022. One hundred and fifty seven (157) neonates were observed over the 72 hours following admission to the Special Care Nursery. Neonates were grouped into those that had triggers (vital signs that fell in the red zone) and a stable group (who had no observations in the red zone). Of the 157 patients recruited 72 (45.9%) were stable, and 85 (54.1%) had triggers that should prompt a response. Forty seven (55.3%) of the neonates in the trigger group had appropriate interventions. Neonates with observations in the red zone (triggers) were more likely to die in the first 72 hours compared with those with no triggers. Most of the nurse responded positively to the introduction of the chart. Whilst the Neonatal Early warning System is a tool that can be used to identify neonates at risk of clinical deterioration, proper training of its use and knowledge of and appropriate escalation of care are necessary to ensure its benefit.

**Keywords:** Neonatal Early Warning System, Low Middle Income Countries

**INTRODUCTION**:

In 2020, globally there were about 2.4 million newborns who died before the 28th day of life. Neonatal deaths accounted for 47% of all under five deaths [1]. Most of these neonatal deaths are in low to middle income countries [LMIC]. Many deaths result from lack of basic quality care in the first days after birth.

Papua New Guinea (PNG) is a low to middle income country in the Western Pacific Region with an estimated population of 9.9 million people in 2021 [2]. In 2020, the World Health Organization (WHO) estimated the neonatal mortality rate in Papua New Guinea to be 22 per 1000 live births [1]. Trends overall in recent years have shown a steady decline, but this figure remains one of the highest in the Pacific. It is also considerably higher than the target of 12/1000 live births as suggested by the United Nations Sustainable Development Goals [3]. The 2021 Paediatric Annual Mortality and Morbidity report stated a hospital case fatality rate for neonates to be 5.9%. In the same report, recommendations were made to use color coded charts to help identify neonates at risk [4].

Paediatric early warning systems (PEWS) have been used for many years including those designed for use in LMICs [5] but there is limited information of Newborn Early Warning System (NEWS) even in high income countries. A small study from UK reported the importance of a NEWS for detecting early sepsis [6]. An international review published in 2017 found only 4 published systems and these were primarily designed for term or near term infants in postnatal wards [7].

Although there is a color-coded chart formulated by the Paediatric Society of Papua New Guinea for use in children, there is none specifically tailored for use in neonates. There are no published studies in Papua New Guinea on the use of color-coded charts or track and trigger systems.

The aim of this study was to describe the use of a Neonatal Early Warning System adapted from the Plymouth Hospital Neonatal Early Warning System chart [8] in Port Moresby General Hospital (PMGH) Special Care Nursery (SCN).

Our primary objective was to describe neonates who had vital signs in the trigger or red zone and to document the interventions applied and their outcomes at 72 hours. Our secondary objective was to assess the response of nursing staff to the use of the NEWS.

**METHODOLOGY**:

A descriptive observational study was conducted in PMGH, SCN during an eight-week period (1st May 2022 to 30th of June 2022). The color-coded NEWS chart was used to record vital signs (Figure 1). Observations in the red zone were triggers to initiate appropriate interventions such as administration of oxygen or increasing oxygen flow rate, adjusting positioning, initiation of intravenous

antimicrobial therapy or administration of anticonvulsants.

Prior to the study period, a presentation on how to use the Neonatal Early Warning System was given to the nursing staff in the SCN. This included discussion of the interventions required in the event of observations being in the red zone.

Neonates born at PMGH who were identified by the paediatric team on duty as needing admission to the nursery using the standard Special Care Nursery guidelines were included in the study. Babies born in hospitals other than PMGH, who were more than 72 hours old and who had been admitted prior to the study period were excluded, as well as babies who had structural congenital abnormalities of the respiratory and cardiovascular system.

A NEWS observation form was added to the admission charts. Routine vital observations were carried out at normal times as per SCN practice. Vital signs were taken using routine equipment and observations were recorded. A separate data collection form was used to gather the data.

Neonates were followed up for 72 hours following admission to the nursery, and their outcomes at this point were recorded. Characteristics of neonates with their admitting diagnoses were entered into Microsoft Excel 2019 spreadsheets.

The data were summarized using frequency tables and percentages for categorical variables. Odds ratios and p-values were calculated using Open Epi Means and standard deviations for normally distributed continuous variables were calculated using Microsoft Excel 2019. The differences between the groups were assessed by Students unpaired t test. Differences were regarded as significant if the p-value was ≤0.05.

Nurses were asked to provide feedback on the use of the NEWS chart after the end of the study period using a self- designed structured questionnaire incorporating a Likert Scale. Questions were grouped into 4 categories: usability of the form, monitoring and interpretation of abnormal vital signs, management of abnormal vital signs and communication.

**RESULTS**:

A total of 157 neonates were included in the study of whom, 85 (54.1%) had vital signs that fell in the red zone and formed the trigger group whilst 72 (45.9%) had vital signs within the acceptable range and formed the stable group. Each neonate was followed up for the first 72 hours of admission and their outcome recorded.

Details of the neonates in each group is shown in Table 1. The mean birth weights and gestational age in the two groups were similar, $p = 0.27$ and $p = 0.34$, respectively. There was a higher proportion of neonates born by Caesarean section in the stable group than the trigger group ($p = 0.01$).

**Table 1. Baseline characteristics of neonates in each group**

| CHARACTERISTIC | STABLE: (n =72) N (%) | TRIGGER: (n = 85) N (%) |
|---|---|---|
| SEX | | |
| Female | 37 (51.4) | 48 (56.5) |
| Male | 35 (48.6) | 37 (43.5) |
| GESTATIONAL AGE | | |
| Less than 37/40 | 27 (37.5) | 33 (38.8) |
| Greater than or equal to 37/40 | 45 (62.5) | 52 (61.2) |
| Mean (standard deviation) gestational age in weeks | 37.13 (3.02) | 36.62 (3.59) |
| BIRTH WEIGHTS | | |
| Less than 1 kg | 4 (5.6) | 6 (7.1) |
| 1 kg to 2 kg | 19 (26.4) | 29 (34.1) |
| 2 kg to 2.5 kg | 12 (16.7) | 12 (14.1) |
| More than 2.5 kg | 37 (51.4) | 38 (44.7) |
| Mean (standard deviation) birth weight in kg | 2.51 (0.93) | 2.33 (1.03) |
| DELIVERY MODES | | |
| Normal vaginal delivery | 55 (76.4) | 77 (90.6) |

**Table 2. Diagnoses of the neonates in each group**

| DIAGNOSIS | STABLE GROUP (n=72) N (%) | TRIGGER GROUP (n=85) N (%) |
|---|---|---|
| Meconium aspiration syndrome | 2 (2.8) | 17 (20) |
| Prematurity with complications | 7 (9.7) | 22 (25.9) |
| Intrauterine growth restriction with complications | 20 (27.8) | 15 (17.7) |
| Neonatal sepsis (including meningitis and pneumonia) | 25 (34.7) | 25 (29.4) |
| Birth asphyxia - mild | 8 (11.1) | |
| Birth asphyxia – severe * | 1 (1.4) | 6 (7.1) |
| Transient tachypnoea of the newborn | 1 (1.4) | - |
| Subgaleal Haemorrhage | 1 (1.4) | - |
| Jaundice | 7 (9.7) | - |

* With signs of hypoxic ischaemic encephalopathy

There were marked differences in the diagnosis of the two groups, with meconium aspiration syndrome (MAS), prematurity with complications and severe birth asphyxia accounting for 45 (53%) of the 85 neonates in the trigger group. The proportion of neonates with sepsis was similar in the groups (p = 0.48).

The incidence of observations in the red zone in the trigger group babies during the three 24 hour periods is shown in table 3.

Some babies had more than one physiological abnormality

**Table 3. Abnormal observations in the trigger group over the first 72-hours N=85#**

| PHYSIOLOGICAL ABNORMALITIES | 0-23 HOURS N (%) | 24-47 HOURS N (%) | 48-71 HOURS N (%) | TOTAL N (%) |
|---|---|---|---|---|
| Fever | 24 (28.2) | 16 (18.8) | 11 (12.9) | 51 (60) |
| Hypothermia | 20 (23.5) | 10 (11.8) | 11(12.9) | 41 (48.2) |
| Grunting | 9 (10.6) | 0 | 0 | 9 (10.6) |
| Tachypnoea | 9 (10.6) | 4 (4.7) | 4 (4.7) | 17 (20) |
| Bradypnoea | 4 (4.7) | 3 (3.5) | 0 | 7 (8.2) |
| Spasticity / Convulsions | 12 (14.1) | 2 (2.4) | 1 (1.2) | 15 (17.6) |
| Floppy | 3 (3.5) | 1 (1.2) | 0 | 4 (4.7) |
| Dusky / Cyanosis | 31 (36.5) | 10 (11.8) | 3 (3.5) | 44 (51.8) |
| Tachycardia | 4 (4.7) | 0 | 1 (1.2) | 5 (5.9) |
| Bradycardia | 1 (1.2) | 1 (1.2) | 0 | 2 (2.4) |
| Hypoglycemia | 0 | 0 | 0 | 0 |
| Hyperglycemia | 2 (2.4) | 0 | 0 | (2.4) |

**Table 4. Outcomes of patients at 72 hours**

| OUTCOMES | STABLE GROUP (n=72) N(%) | TRIGGERS GROUP (n=85) N(%) |
|---|---|---|
| Still unstable after 72 hours | 11 (15.3) | 32 (37.7 ) |
| Rooming with mother | 22 (30.6) | 17 (20) |
| Transferred to post-natal ward | 17 (23.6) | 7 (8.2) |
| Died | 7 (9.7) | 27 (31.8)) |
| Discharged | 11 (15.3) | 2 (2.4) |
| Absconded | 1 (1.4) | 0 |
| Lost to follow up | 3 (4.2) | |

Cyanosis was the most common trigger in the first 24 hours. Both high and low temperatures in the trigger zones were recorded in all three time periods and fever was the most observed trigger overall. Only 47 (55.3%) of the 85 patients in the trigger group had an appropriate intervention carried out. The outcome of the neonates at 72 hours after admission is shown in Table 4.

Neonates with triggers were 4.3 (1.7-12.6) times more likely to die than those without (p=0.001).

Ten of the 27 babies who died in the trigger group had hypothermia for which adequate intervention was not initiated. The 7 babies who died in the stable group were extremely low birth-weight and/or preterm and all died suddenly within 12 hours of admission. There was no significant difference in mortality in the neonates in the trigger group who did and who did not receive intervention (17/47 vs 10/38 p = 0.46).

**Nursing staff responses to the introduction of the NEWS.**

The responses of the 13 nursing staff with differing levels of training and experience in neonatal care (6 general nurses, 2 community health workers and 5 specialty nurses) who provided feedback are shown in Table 5. All but one gave positive responses.

**Table 5. Nursing staff responses to the introduction of the NEWS (n=13) N (%)**

| USABILITY OF THE FORMS | STRONGLY DISAGREE | DISAGREE | AGREE | FULLY AGREED |
|---|---|---|---|---|
| I find the NEWS observation form user friendly | 1 (7.7) | | 2 (15.4) | 10 (76.9) |
| I find the NEWS observation form can help in identifying sick neonates quickly | 1 (7.7) | | | 12 (92.3) |
| I understand the color-coded lines | | | 2 (15.4) | 11 (84.6) |
| I find the forms and font size clear enough for A4 size | 1 | | | 12 |
| **USAGE OF EQUIPMENT, AND UNDERSTANDING VITAL SIGNS** | | | | |
| I understand how to correctly use a digital thermometer to measure temperature | | | | 13 |
| I understand how to correctly use a pulse oximeter to get oxygen saturation and pulse rate | | | | 13 |
| I understand how to use a blood glucose machine to measure sugar levels | 1 | | | 12 |
| I understand how to measure the respiratory rate of neonates | 1 | | | 12 |
| I understand the normal ranges for each of the vital signs | 1 | | | 12 |
| I can easily attend to a neonate with fever | 1 | | | 12 |
| I can easily manage a neonate with hypothermia | 1 | | | 12 |
| **MANAGEMENT OF A NEONATE WITH AN ABNORMAL VITAL SIGN** | | | | |
| I can easily manage a neonate with cyanosis and respiratory distress | 1 | | 2 | 10 |
| I can easily manage a neonate that has an apnoeic attack | 1 | | | 12 |
| I know what to do immediately when a neonate is fitting | | | | 13 |
| I know what to do when a neonate is not feeding properly | 1 | | 1 | 11 |
| I can easily manage a child who has hypoglycemia | 1 | | 1 | 11 |
| I can manage a neonate who has had a cardiorespiratory arrest | 1 | | 2 | 10 |
| **COMMUNICATION** | | | | |
| I feel I am able to discuss sick neonate with my senior colleagues easily | | | 2 | 11 |
| In an emergency, I am able to get through to the on-call resident easily from nursery | | | 2 | 11 |
| In an emergency, I am able to get through to the on-call medical officer easily from nursery | | | 2 | 11 |
| When needed I am able to get through to the on-call Specialist Paediatrician easily from nursery | | | 3 | 10 |

## DISCUSSION:

This study has shown that the NEWS can be used as a tool to aid in the diagnosis and management of neonates at high risk of mortality admitted to our SCN. It is likely that similar NEWS will be of benefit in situation similar to ours in which high tech monitoring and intervention is not available. Of the 13 nursing staff that participated in this study 12 (92.3%) found the chart easy to use. They were confident in using the digital thermometers, pulse oximetry and blood glucose machines, and interpreting the abnormal values. However, our study found that whilst babies whose observations fell in the red zone of the chart were 4 times more likely to die than those with no triggers, only 47 (55.3%) of these 85 high risk babies received appropriate escalation of care. Ten (27.0%) of the 27 babies who died in the trigger group had hypothermia recorded for which no intervention was initiated.

Hypothermia is recognised as a major risk factor for neonatal mortality even in tropical climates [9]. Interventions such as administering oxygen and adjusting flow to maintain adequate oxygenation, correcting hypothermia, administration of intravenous appropriate antibiotics, maintaining normal blood glucose levels with intravenous dextrose or carefully administered nasogastric feeds and ensuring correct positioning are interventions that can and should be done in such settings as ours without highly technical and complex and expensive neonatal intensive care facilities. Similar scoring systems have been trialed and found effective in other similar settings [10].

Our study has demonstrated that NEWS is an important tool for improving quality of care for our neonates but that its introduction requires initial training of the SCN staff, encouragement and support for its use and regular assessment of its impact.

**LIMITATIONS**:

This study was conducted over a short time period. Further study should assess the use of NEWS over a prolonged period to determine sustainability.

Precise causes of death could not be determined but many deaths were thought to be unavoidable.

**CONCLUSIONS**:

Despite its limitations, this study has provided an insight on the use of a track and trigger system to escalate care on high risk neonates. It confirms that the use of a NEWS is feasible in our setting but maximizing its effect requires training, reinforcement, and regular assessment.

Acknowledgement: We are grateful to the nursing staff of the SCN for taking part in this study.

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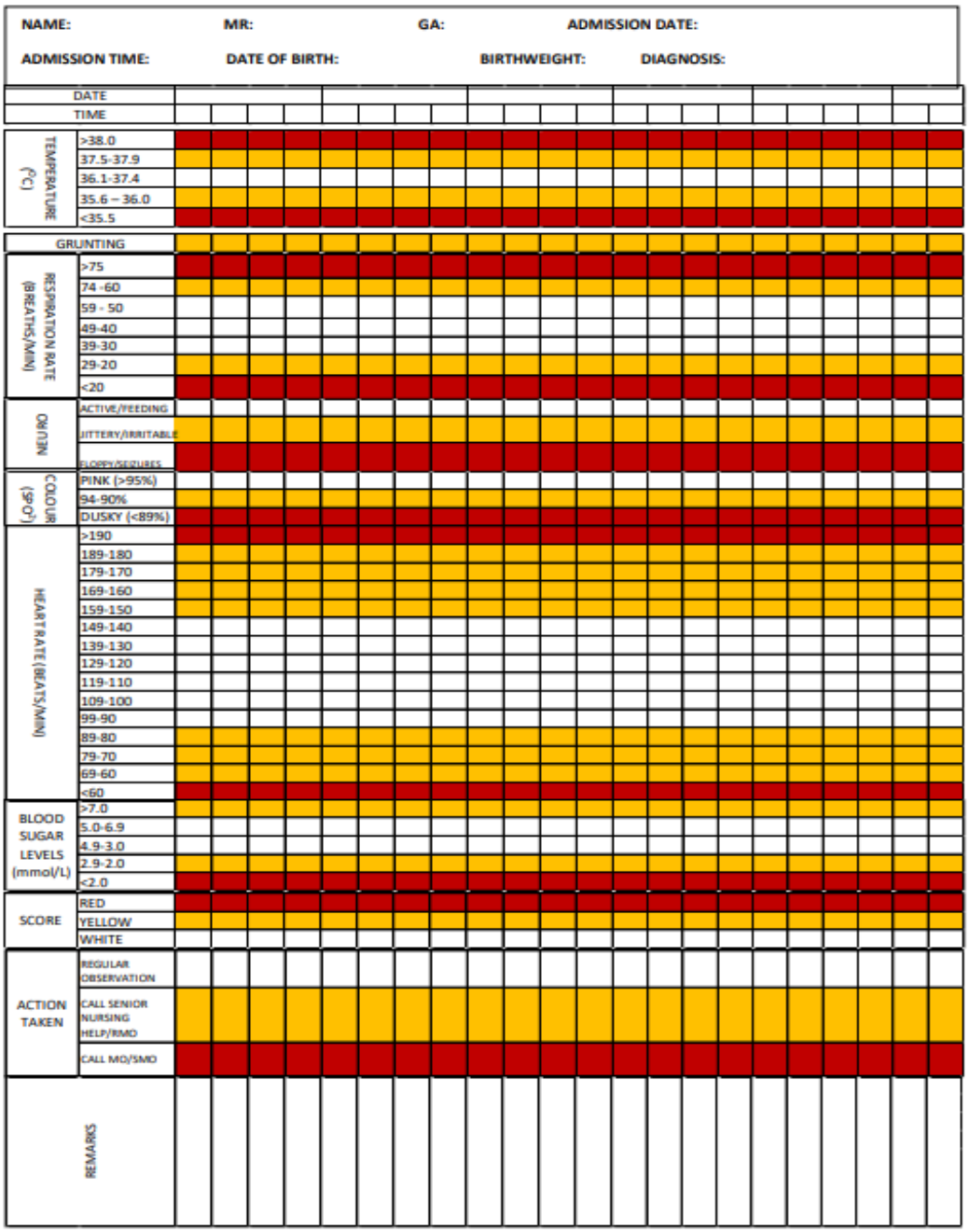
Figure 1. Neonatal Monitoring and Response Chart

Derived from Plymouth NEWS: https://www.infantjournal.co.uk/pdf/inf_034_ris.pdf



# INTERVENTIONS IMPLEMENTED TO REDUCE BACTERIAL CONTAMINATION IN BLOOD PLATELETS AT THE NATIONAL BLOOD TRANSFUSION SERVICES IN GUYANA

Running title: Reduction of Platelet Contamination

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**ABSTRACT**:

This study aimed to determine the prevalence of bacterial contamination in donor-collected platelets components using the diversion pouch integrated blood collection system at the National Blood Transfusion Services, Guyana. This was a single-site cross-sectional, comparative study that was carried out at the blood bank from July 2022 to August 2022. A total of 70 platelet concentrates were collected, 35 samples were from donors swabbed with isopropyl alcohol and the other 35 samples were from donors swabbed with a combination of hand scrub, iodine and isopropyl alcohol. Gram staining, culture, and subculture were done in Trypticase Soy Broth, blood agar and eosin methylene blue agar, Mac Conkey agar, and Chocolate agar. Of the 70 samples tested, 7 (10%) samples had contamination. Of the 7 contaminated samples, 5 samples were contaminated with Gram-negative bacilli, 1 sample each with Gram-negative cocci and Gram-positive cocci. The occurrences of bacterial contamination of platelets were significantly lower when utilizing the combination of hand scrub, iodine, and isopropyl alcohol (1 in 35) in comparison to the usage of isopropyl alcohol alone (6 in 35), as well with diversion pouch ($p \leq 0.05$). A diversion pouch blood collection system in combination with an aseptic method of iodine, isopropyl alcohol, and a hand scrub is an efficacious method in reducing bacterial contamination in platelets.

Keywords: bacterial contamination, platelets, diversion pouch

*Submitted: June 2023; Accepted: August 2023*

**INTRODUCTION**:

Globally, people are impacted by the serious public health problem of microbial contamination of donor blood and other blood-related derivatives. The existence of bacteria in blood or blood derivatives collected and/or prepared for transfusion is known as bacterial contamination of donated blood. Microbial contaminants, such as bacteria, should be absent from the blood that is ready to be transfused [1]. This necessitates the collection and processing of blood in an aseptic manner. Blood donation systems have generally implemented the redirection of the first flow of blood into a pouch as a way to avoid whole-blood unit contamination from bacteria. Thus, the diversion pouch could be used as an alternative to venipuncture for blood collection [1].

In transfusion medicine, bacterial transmission is still a major issue. This is not a recent issue; the first report of bacterial transfusion transmission from a blood component was documented in 1941, more than 60 years ago. Since the 1970s, significant progress has been achieved in improving the viral threat to the blood supply. Bacterial contamination remains the most common microbiological cause of transfusion-associated illnesses [2]. Bacterial contamination of derivatives from blood, particularly platelets, is the most common infection risk of blood transfusion, occurring in around 1 out of every 2000-3000 platelet infusions [3]. The most common bacterial contamination of blood products is by gram-positive bacteria often found on the skin, such as *Staphylococcus* epidermidis or *Staphylococcus aureus*, *Corynebacteria spp*, and *Bacillus spp* [3]. This contamination occurs as a result of bacteria on the skin moving through the collecting needle into the blood. Gram-negative bacteria are common in the gastrointestinal tract's natural flora and occur when blood is drawn from donors who have bacteria in their circulation but are asymptomatic. Examples include *Acinetobacter*, *Klebsiella*, and *Escherichia coli* [3]. Garraud and Tissot looked at the survival modes of bacteria in the various fractions of blood, and it was proven that contamination of blood components may be traced back to contamination during the collection, preparation, and pooling methodologies. As well as contamination in collecting bags and blood product storage period [4].

The National Blood Transfusion Service (NBTS) collects voluntary blood donations (10,000 units of whole blood yearly) at several places around Guyana during blood drives, resulting in minimally standardized sample collection techniques, which could put blood components at risk of contamination. Despite standard operating protocols being in force at blood banks and hospitals to minimize microbiological contamination of blood bags in storage, contamination from bacteria does occasionally

occur [5], [6]. Bacterial contamination is more common in platelet concentrates than in RBC components, since bacteria may live and multiply at the temperature levels used for PLT (20-24 degree Celsius), but not for RBC (1-6 Degrees Celsius). Because of the growing knowledge and clinical importance of bacterial contamination of blood components, standards have been implemented to reduce bacterial infections [3]. Even after transfusion, and especially after the transfusion of platelets, bacterial sepsis remains a serious concern. Bacterial contamination of whole blood is a result of natural skin flora. Skin disinfection before venipuncture is critical for lowering the risk of post-transfusion infection [7]. Cleansing the arm with detergent and water makes it easy to get rid of the temporary flora. After washing the skin, blood culture findings reveal 2% to 6% rate of positive cultures [7].

**METHODOLOGY**:

Platelet donor samples were obtained from the National Blood Transfusion Service and transported to the University of Guyana Lab, where they were analyzed throughout July and August 2022. A total of 70 platelet components were collected, stored appropriately, and transported to the University of Guyana's Laboratory for processing. A prospective and convenient sampling method was used. All platelet concentrates were packed and placed in an ice cooler. The ice cooler was equipped with a thermometer for temperature control (ideal temperature: 22 degrees Celsius). The platelet components were then transported within two days after collection from the National Blood Transfusion Bank to the University of Guyana Laboratory.

In the lab, 5 ml of the platelet concentrates were injected into vials containing 50 ml of Trypticase Soy Broth, after which they were placed in the incubator and then observed for approximately 7 days for any signs of turbidity. The vials with turbidity were gram-stained and then sub-cultured on MacConkey agar, Chocolate agar, and Blood agar. As opposed to Chocolate agar, which was incubated anaerobically at 37°C for 48 hours, MacConkey and Blood culture plates underwent aerobic incubation for 24-48 hours. Upon detection of growth, a gram stain was done and checked under the microscope to differentiate between gram-positive and gram-negative organisms [8].

Data were entered into an Excel spreadsheet and analyzed using SPSS Version 21.0 Software. Data was graphically presented using Bar charts, Pie charts, and Tables. Permission was given by the Institutional Review Board (IRB# 027/2022) and the director of the National Blood Transfusion Service before commencing this research. All patient information was kept confidential.

**RESULTS**:

A total of 70 samples were collected in the study, 35 of which were exclusively from donors whose phlebotomy sites had been cleaned with

isopropyl alcohol. Another 35 samples were collected from donors whose arms had been scrubbed with liquid soap before being cleaned with isopropyl alcohol and iodine. Table 1 shows the summary of the result obtained. Out of the 70 samples that were taken, 7 (or 10%) showed signs of growth, while 63 (or 90%) showed no growth. Gram-negative bacilli (5), Gram-negative cocci (1), and Gram-positive cocci (1) were the different bacterial types identified.

Table 2 shows that when the donors were swabbed with isopropyl alcohol, growth was seen in 6 (17.1%) of the 35 samples; therefore, no growth was seen in the remaining 29 (82.9%) samples. With a combination of both iodine and isopropyl alcohol and hand scrub, growth was seen in 1 (2.9%) sample from a total of 35; the remaining 34 (97.1%) samples had no growth.

Table 1: Overall findings of the study

| Variables | N (%) | p - value |
|---|---|---|
| Type of swabbing | | |
| Isopropyl alcohol | 35 (50%) | |
| Iodine + Isopropyl alcohol | 35 (50%) | |
| Presence of bacteria | | |
| Growth | 7 (10%) | |
| No growth | 63 (90%) | p≤ 0.05 |
| Type of bacteria | | |
| Gram negative bacilli | 5 (7.1%) | |
| Gram negative cocci | 1 (1.4) | |
| Gram positive cocci | 1 (1.4) | |
| No growth | 63 (90.0) | p≤ 0.05 |

Table 2: Percentage of growth with different Aseptic Techniques used

| Presence of bacteria | Type of swabbing | | |
|---|---|---|---|
| | Isopropyl alcohol | Hand scrub + Isopropyl alcohol + Iodine | p - value |
| Growth | 6 (17.1%) | 1 (2.9%) | |
| No growth | 29 (82.9%) | 34 (97.1%) | 0.04 |

**DISCUSSION**:

The study evaluated the prevalence of bacterial contamination in platelet components using diversion pouches at the National Blood Transfusion Service. First, before phlebotomy, cleaning the blood collection area is an essential step in reducing the likelihood of spreading pathogens during transfusion. It has been demonstrated that the diversion pouch is crucial for minimizing bacterial contamination. Diverting the first 10-15 ml of blood is an effective means of mitigating the percentage of contamination with bacteria [9]. The most frequent cause of gram-negative bacteria such as *E. coli* is occult bacteremia [10]. Consequently, all gram-negative organisms should be viewed as potentially dangerous to the donor's health. The presence of fever, rigors, and hypotension is indicative of the importance of other characteristics of the organisms, such as the strain's virulence, in transfusion-associated sepsis [9]. Before venipuncture, epidermal cleaning is essential for reducing the incidence of post-transfusion infection. As mentioned earlier, human skin has two different bacterial floras: transient and residential [10].

Thyer and fellow co-authors found that when a needle is inserted into the skin to collect blood, results in a passage of live bacteria or the transfer of bacteria from tiny pedicle flaps created by the needle into the bags that collect [11]. A skin surface that has been scarred by prior donations [12] makes it considerably more challenging to obtain thorough decontamination of the region [13].

The first part of blood collection should be diverted to collateral bags for biological validation testing of the unit. This prevents the first inflow of blood-carrying pathogens or epidermal shards from the donor to infiltrate the collecting bag and infecting it. The transfusion community and the FDA have taken many steps to reduce the risk of microbial contamination of transfusion products during the period of collection. More efficient sanitation of the phlebotomy site is an apparent first step. The FDA has spoken out concerning the need for appropriate antibacterial implementation and scar tissue complications. To sanitize the collecting locations, more efficient disinfectants have been advocated, and the practice of inefficient antibacterial agents such as green soap has been deterred [14]. In tests involving hospitalized patients, however, FDA found that some skin disinfection treatments utilizing isopropyl alcohol accompanied by an infusion of iodine, which had been claimed to be better than those using povidone-iodine, were ineffective. Once the venipuncture needle punches out an epidermal lump as it penetrates through the skin, sterilization of the surface of the skin is limited. This unsterilized epidermis component can be taken into the collection of blood and developed [11].

Cold temperatures might reduce the rapid growth of microorganisms in transfusion items during storage. This is a typical procedure for red blood cells since it inhibits the development of the majority of bacterial species and lowers the possibility of sepsis caused by transfusion. Platelets, inversely, lose their ability to function properly when kept at low temperatures. Short exposure to cold can cause irreparable platelet damage that severely reduces their capacity to circulate [15].

Transfusion with blood components that are contaminated with exogenous bacteria or other agents can cause fatal complications [16]. To reduce pathogen contamination, a series of particular activities and processes known as aseptic methods are carried out under strictly controlled settings. Making use of such methods is vital because they safeguard both the donor and patient from infection and stop the spread of infections. At times, cleaning (removal of dirt and other impurities), sanitizing (reduction of microorganisms), or disinfecting (removal of the majority of bacteria but not those with high resistance) techniques are insufficient to prevent infection [16]. Through interaction with the environment, people, or equipment, pathogens can infect the patient. Any therapeutic environment may benefit from utilizing aseptic techniques, which is why they should always be used to preserve asepsis, the absence of harmful organisms, in the clinical context [16].

Conclusion & Recommendations:

In transfusion medicine, bacterial transmission continues to be a major concern. It can be concluded based on our findings that the contamination of platelet concentrates via the diversion pouch occurs at a significantly lower proportion when compared to that of the traditional collection bag. Under this study, the most efficient way to disinfect skin is to use a hand scrub in conjunction with isopropyl alcohol and iodine.

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# LABORATORY FINDINGS IN THE CEREBROSPINAL FLUID OF CHILDREN WITH SUSPECTED MENINGITIS ADMITTED TO PORT MORESBY GENERAL HOSPITAL: A RETROSPECTIVE STUDY

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**ABSTRACT:**
Whilst the advent of vaccination against the common causative pathogens and improved hygiene have reduced the incidence in high income countries, bacterial meningitis remains a common cause of childhood morbidity and mortality in low- and middle-income countries. Confirmation of the diagnosis depends on laboratory examination of cerebrospinal fluid. The aim of this study was to determine the incidence of laboratory confirmed meningitis in children diagnosed with clinical meningitis admitted to Port Moresby General Hospital. The records of cerebrospinal fluid findings from children aged less than 13 years presenting with suspected meningitis were examined retrospectively. Macroscopy, microscopy, gram stain and bacterial culture data were gathered from the microbiology log. Descriptive statistics, were used to analyse the data. All 906 cerebrospinal fluid samples were examined macroscopically while 9 (1%) had microscopy only and 897 (99.0%) had microscopy and culture performed. A laboratory-confirmed diagnosis was possible for 412/906 (45.5%) children, but a definite pathogen was identified in only 16/412 (3.9%). *Streptococcus pneumonia* was the leading isolate followed by *Neisseria meningitides.* The majority of children who had laboratory confirmed diagnoses were less than 2 years old. Meningitis is still a common cause of admission, and *Streptococcus pneumonia*, and *Neisseria Meningitides* are important pathogens in children in Papua New Guinea. There is an urgent need to improve routine vaccination coverage.

**Keywords**: Cerebrospinal fluid, Bacterial pathogens, Meningitis, Children, Port Moresby, Papua New Guinea

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## INTRODUCTION:

Bacterial meningitis is still a significant cause of mortality and morbidity among children worldwide [1, 2]. Young children living in communities that have low socioeconomic status and poor medical infrastructure are at highest risk of dying [3, 4]. Meningitis can be caused by viruses, fungi or bacteria but bacteria are associated with the highest frequency and a high morbidity and mortality.

Studies from previous decades showed that *Streptococcus pneumoniae*, *Haemophilus*

*influenza B* and *Neisseria meningitides* were the leading causes of bacterial meningitis in young children under five years of age [2, 5-7]. The clinical features of bacterial meningitis vary but commonly children develop neck stiffness, bulging fontanel, positive Kernig's and Brudzinski signs, fever, altered conscious level, vomiting, convulsion and purpuric rash [8, 9]. Both clinical and laboratory diagnosis are often made more difficult following the administration of antibiotics before presentation to the hospital. The diagnosis of bacterial meningitis cannot be clearly distinguished from other common childhood illnesses such as encephalitis and other severe bacterial infections because their clinical features overlap. Laboratory examination of cerebrospinal fluid is the only way to confirm the diagnosis [1, 10-14]. Cerebrospinal fluid (CSF) culture and gram staining are the gold standard and widely used in low resource settings. Latex agglutination and Polymerase Chain Reaction (PCR) can also be used but are expensive. Each of the CSF examination techniques has its own challenges in terms of its predictive assumptions, availability, affordability, expertise and the effect of prior antibiotic treatment [15].

Prevention and effective treatment are key to reducing the incidence, morbidity and mortality from bacterial meningitis. The introduction of effective conjugate bacterial vaccines against, *Streptococcus pneumoniae* and *Haemophilus influenza* and the availability of third generation cephalosporin, has contributed to reduction in under five mortality in many countries [10, 16-18].

The aim of this study was to determine the proportion of laboratory confirmed cases of meningitis in children with an initial diagnosis of clinical meningitis who were admitted to Port Moresby General Hospital.

**METHODOLOGY**:

Study design and setting: This was a retrospective analysis of laboratory records from 2017 to 2021 at Port Moresby General Hospital microbiology section of the pathology laboratory. Purposive sampling was used to collect the data, on children aged 13 years and younger who were admitted with the clinicians' diagnosis of suspected meningitis and who underwent lumbar puncture.

Data collection strategy: We collected information from the CSF sample registry. The variables obtained were, age, year of collection, macroscopic findings, microscopic findings-differential white cell counts, red blood cells, gram stain, Indian ink, and culture. Data on protein and sugar, and acid-fast staining (AFB) for tuberculosis (TB) was not recorded in the microbiology section.

In the present study laboratory confirmed meningitis was defined as follows:

- for infants > 1 month and children up to 13 years, presence of polymorphs, lymphocytes or positive gram stain or culture.

- for neonates: polymorphs > 10 in the first week and 0 if aged more than 1 week [8, 19]. Lymphocytes > 20 in the first weekof life, and > 6 in more than 1 week of age.

Statistical analysis: relevant data from samples were abstracted and entered into Microsoft Excel version 2013, and analysed using IBM SPSS statistics software version 22. Median, interquartile ranges, frequency and percentages were used to describe the data.

Ethical approval was obtained from the School of Medicine and Health Sciences ethics committee and permission granted by Port Moresby General Hospital to perform data collection.

**RESULTS**:

Records of 906 cerebrospinal fluid samples from children with an admitting diagnosis of meningitis were examined. Of the 906, 57.7% (523) were males and 42.3 % (383) were females. The median age was 5 months (Interquartile range 2-15 months). The majority of all the children were aged 12 months and below (Table 1). From the 5 years data, there was almost equal distribution of cases with normal CSF and laboratory confirmed diagnosis (Table 2).

**Table 1. Age groups of children with CSF (n = 906)**

| Age groups | N (%) |
|---|---|
| **< 1 month** | 127 (14) |
| **1-12 months** | 529 (58.4) |
| **> 12 months-60 months (5 years)** | 158 (17.4) |
| **> 60 months (5 years)** | 91 (10) |
| **Unknown** | 1 (0.1) |

CSF examination findings:

Macroscopy. Six hundred and seventy-seven (74.7%) of the 906 samples were reported as clear fluids with no clots, 4 (0.4%) grossly blood stained with no clots, 81 (8.9%) slightly blood stain with no clots, 92 (10.2%) Xanthochromic with no clots, 2 (0.2%) grossly turbid and 50 (5.5%) slightly turbid.

Microscopy culture and sensitivity. Of the 906 samples, 9 (1.0 %) have microscopy only, and 897 (99.0%) had microscopy, culture and sensitivity performed. There was no growth on culture of 881 (98.2%) of the 897 samples and species were identified in 16 (1.8 %) (Table 3).

**Table 2. Laboratory incidence of meningitis over five-year periods (2017-2021)**

| **Diagnosis (**n = 906) | **N (%)** |
|---|---|
| **Normal CSF** | 494 (54.5) |
| **Laboratory confirmed** | 412 (45.5) |
| **Isolation rate:** | 16 (1.8) |

| **Proportion per year n = 906** | **N (%) (n = 906)** | **Normal CSF (n = 494) N (%)** | **Abnormal CSF (n = 412) N (%)** |
|---|---|---|---|
| **2017** | 151 (16.7) | 85 (17.2.) | 66 (16.0) |
| **2018** | 134 (14.8) | 84 (17.0) | 50 (12.1) |
| **2019** | 168 (18.5) | 87 (17.6) | 81 (19.7) |
| **2020** | 239 (26.4) | 126 (25.5.) | 113 (27.4) |
| **2021** | 214 (23.6) | 112 (22.7) | 102 (24.8) |

**Table 3. Total frequency of pathogens identified in CSF samples over the five years period (2017 – 2021), n =897**

| **Pathogens Identified** | **Counts: N (%)** |
|---|---|
| **Streptococcus. Pneumonia** | 6 (0.7) |
| **Neisseria. Meningitides** | 2 (0.2) |
| **Hemophilus. Influenza** | 1 (0.1) |
| **Escherichia. Coli** | 1 (0.1) |
| **Pseudomonas. Aeruginosa** | 1 (0.1) |
| **Enterobacter. Sakazakii** | 1 (0.1) |
| **Citrobacter. Freundii** | 1 (0.1) |
| **Acinetobacter. Anitratus** | 1 (0.1) |
| **Streptococcus Species** | 1 (0.1) |
| **Cryptococcus. Neoformans** | 1 (0.1) |
| **Total** | 16 (1.8) |

Gram stain was performed on all (906) samples. The results show that 792 (87.4%) had no organism seen, 14 (1.5 %) had organism seen, and 100 (11.0 %) has only pus seen. There were

16 bacterial pathogens that were identified by culture, including the 14 that were seen on gram staining.

Differential white cell count. Of the 906 samples, polymorphs were seen in 319 (35.2%), 397 (43.8 %) had lymphocyte presence and 501 (55.3 %) had red blood cell presence. The median polymorph count was 8 (Interquartile range 2-40, range 0-9220), lymphocyte 8 (Interquartile range 2-32, range 1-3100) and red blood cell 21 (Interquartile range 4-205, range 0-44800).

Indian ink and cryptococcal antigen. Of the 906 samples, only 1 (0.1%) was Indian ink and cryptococcal antigen test positive.

**DISCUSSION**:

Bacterial meningitis remains a disease of concern in children because of its high mortality and morbidity. Early and accurate diagnosis and prompt treatment is required to avoid death and prevent complications. For the effective and prompt treatment of bacterial meningitis, knowledge of the clinical characteristics, aetiology, and antimicrobial susceptibility of the causative organisms are crucial. CSF examination is needed to establish the diagnosis. Delays in diagnosis and treatment can lead to long-term problems such as hearing loss, learning disabilities, hydrocephalus, and death [7, 17, 20].

The diagnosis of meningitis in children is challenging because symptoms and signs are not specific. Lumbar puncture and examination of CSF is the practical way to make a diagnosis in most of the low–and-middle income countries (LMIC). Since the risks of missing the diagnosis are considerable, it is inevitable that some children without meningitis may have a lumbar puncture and normal CSF. In our study more than half of the children who were admitted with suspected meningitis had normal CSF, and slightly over 45% had laboratory confirmed diagnosis. Whilst children who do not have meningitis are often subjected to lumbar puncture, children with meningitis may have contraindications to lumbar puncture, such as evidence of raised intracranial pressure or being dangerously sick.

Because of the severity of the disease, children suspected of having meningitis are often started on antibiotic treatment before there is an opportunity for a safe lumbar puncture. This adversely affects the likelihood of obtaining a positive bacterial culture and the effect on pleocytosis makes the diagnosis of aseptic viral and tuberculous meningitis difficult [21].

In this study, the highest number of cases of suspected meningitis was in 2020. This may be related to the Covid-19 pandemic, which had an adverse impact on routine vaccination coverage, health seeking behaviour, and the availability of health services.

Microbial culture and identification remains the gold standard for diagnosis of bacterial meningitis. Only 16 of the CSF samples grew bacteria on culture and of these six were Streptococcus pneumoniae and two were

Neisseria meningitides. Studies from South Asia, PNG and Portugal reported similar CSF culture findings [1, 7, 10, 12-14, 22]. Other bacteria capable of causing meningitis such as Group B streptococcus which, since the introduction and high vaccination coverage of Haemophilus influenza B (HiB) and pneumococcal vaccines are the leading causes in developed countries are less frequent in LMICs like PNG, where vaccination coverage is poor [23].

The highest incidence and mortality of bacterial meningitis is usually in infants and young children. The disease is to a large extent preventable by vaccination with routine vaccines. Vaccination coverage in PNG is one of the lowest in the world with less than 40% of eligible children receiving the third dose of HiB and pneumococcal vaccines [24]. Improving coverage should be a national priority.

## CONCLUSION:

Meningitis remains a common cause of admission of children aged less than five years of age in PNG. Streptococcus pneumoniae and Neisseria meningitides were the major bacterial pathogens in the present study. Major efforts are required to improve routine vaccination coverage in PNG.

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# AUDIT OF TREATMENT FOR INPATIENTS WITH COVID-19 INFECTIONS ADMITTED UNDER GERIATRIC MEDICINE

Short Running Title: COVID-19 treatment audit Brunei

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**ABSTRACT**:
Older people with COVID-19 infections are at high risk of adverse outcomes, such as hospitalization and death. Initially, all patients with COVID-19 infections were admitted into a designated isolation hospital. After the second wave, these patients could be admitted to any hospital in specific COVID-19 wards, thus all clinicians had to be familiar with the COVID-19 treatment guidelines. This was a retrospective review of electronic medical records for patients admitted under Geriatric Medicine in RIPAS Hospital with COVID-19 infections between 1st April 2022 and 30th September 2022. The local guidelines recommended intravenous remdesivir for patients with risk factors for complications if they presented within a week of symptom onset, as well as dexamethasone and venous thromboembolism prophylaxis. Compliance to these guidelines were audited. Among the 41 patients, approximately two-thirds were wheelchair or bedbound, while more than 40% were fully dependent. Almost one in five passed away in hospital. The compliance rate of treatment with remdesivir was 82.9%, while among the oxygen dependent patients, treatment with dexamethasone and fondaparinux were 88.2% and 70.9% respectively. While there appears to be a relatively high rate of compliance with COVID-19 management guidelines in older people admitted to RIPAS Hospital, there is still some room for improvement, given that older people are at high risk of poor outcomes with COVID-19 infections.

**Keywords**: COVID-19, dexamethasone, geriatrics, remdesivir, treatment
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## INTRODUCTION:

As of 28th February 2023, there were 278698 confirmed cases and 150 deaths due to COVID-19 infections in Brunei Darussalam [1]. In older people, COVID-19 infections are associated with adverse outcomes, such as hospitalization and death. Older people also tend to present atypically with delirium or neurological symptoms and without fever, contributing to a delay in diagnosis and treatment [2].

When the first local case in Brunei was reported on 9th March 2020, all COVID-19 infections were admitted into the designated isolation hospital and community isolation centres [3]. After the second wave started on 7th August 2021, it became clear that it was not possible to maintain a zero-COVID strategy with the newer transmissible COVID-19 variants such as Delta and Omicron strains. Those with mild symptoms were required to self-isolate at home. However, patients with COVID-19 infections requiring hospital admission (for COVID or non-COVID related indications) would be admitted to the nearest hospital and isolated in designated COVID-19 wards [4]. This meant that clinicians in all hospitals had to be able to assess and manage COVID-19 infections and the associated complications. This was particularly relevant for the Geriatric Medicine team, as older people had a higher risk of contracting COVID-19 and develop complications [2].

The COVID-19 treatment algorithm developed by the national COVID-19 clinical management team was an important reference [5]. In the clinical pathway, patients are categorized based on disease severity: Category 1 (C 1) are asymptomatic, Category 2 (C 2) are symptomatic but do not have clinical or radiological evidence of pneumonia, Category 3 (C 3) have COVID-19 associated pneumonia but do not require oxygen, Category 4 (C 4) are oxygen dependent, while Category 5 (C 5) require intubation and ventilatory support [4,5]. Risk factors for complications included unvaccinated or incompletely vaccinated patients, age 60 years and older, immunocompromised due to diseases or treatment, cancer, obesity, and presence of chronic diseases such as heart failure, respiratory disease, liver disease, renal disease and diabetes mellitus [5].

Based on the guidelines, C 1 to C 3 severity cases presenting within 7 days of symptom onset and have risk factors for complications should be treated with intravenous remdesivir for 3 days. Patients with C 4 or deteriorated and required oxygen during the admission should receive dexamethasone and prophylactic anticoagulation in addition to intravenous remdesivir [5]. Given the concerns regarding potential poor outcomes of older people with COVID-19 infections, the compliance to the treatment guidelines were audited.

**METHODS:**

All patients admitted under Geriatric Medicine in RIPAS Hospital with confirmed COVID-19 infections (positive reverse transcription polymerase chain reaction RT-PCR for the SARS-CoV-2 virus) between 1st April 2022 and 30th September 2022 were included. The electronic

health records for these patients were retrospectively reviewed for the following: patient demographics including age and gender, mobility and function in activities of daily living, comorbidities, COVID-19 vaccination status, severity category on admission, whether they were administered remdesivir, dexamethasone or fondaparinux, and outcomes including length of stay and inpatient mortality.

**RESULTS:**

There were 41 patients identified. Median age was 80 years (range 77 to 94 years). Approximately two-thirds were wheelchair or bedbound, while more than 40% were fully dependent on activities of daily living. Table 1 summarises the baseline characteristics of the patients.

Table 1: Baseline characteristics of patients

| **Demographic** | **N (%)** |
|---|---|
| **Gender**: | |
| Male | 19 (46.3%) |
| Female | 22 (53.7%) |
| **Mobility:** | |
| Independent | 10 (24.3%) |
| Walking Aid | 5 (12.2%) |
| Wheelchair-Bound | 13 (31.7%) |
| Bed-bound | 13 (31.7%) |
| **Activities of Daily Living**: | |
| Independent | 9 (21.9%) |
| Semi-Independent / Requires Assistance | 14 (34.1%) |
| Fully dependent | 18 (43.9%) |
| **Co-morbidities**: | |
| Hypertension | 39 (95.1%) |
| Dementia | 20 (48.8%) |
| Diabetes Mellitus | 20 (48.8%) |
| Stroke | 12 (29.3%) |
| Pulmonary Disease | 11 (26.8%) |
| Renal Failure | 10 (24.4%0 |
| **Vaccination Status**: | |
| Unvaccinated | 4 (9.8%) |
| 1 dose | 0 (0%) |
| 2 doses | 23 (56.1%) |
| 3 doses | 14 (34.1%) |

Table 2 shows the treatment administered stratified by severity staging at the time of presentation. As all patients admitted under Geriatric Medicine were considered high risk (at least one risk factor of age 60 years and older), they should be administered intravenous remdesivir. This was given in 34 (82.9%) of patients. For those who are oxygen dependent (C4), they should also be administered dexamethasone and fondaparinux. This was done in 15 (88.2%) and 12 (70.9%) of the 17 Category 4 patients respectively.

Table 2: Treatment administered stratified by severity at the time of presentation

| Treatment Modality | Administered to patient? | C 1 (n=2) | C 2 (n=13) | C 3 (n=9) | C 4 (n=17) | Total (n= 41) |
|---|---|---|---|---|---|---|
| Remdesivir | Yes | 1 (50%) | 10 (76.9%) | 8 (88.8%) | 15 (88.2%) | 34 (82.9%) |
| | No | 1 (50%) | 3 (23.1%) | 1 (11.1%) | 2 (11.8%) | 7 (17.1%) |
| Dexamethasone | Yes | 1 (50%) | 1 (7.7%) | 2 (22.2%) | 15 (88.2%) | 19 (46.3%) |
| | No | 1 (50%) | 12 (92.3%) | 7 (77.8%) | 2 (11.8%) | 22 (53.7%) |
| Fondaparinux | Yes | 0 (0%) | 5 (38.5%) | 7 (77.8%) | 12 (70.6%) | 24 (58.5%) |
| | No | 2 (100%) | 8 (61.5%) | 2 (22.2%) | 5 (29.4%) | 17 (41.5%) |

Table 3: Number of patients classified according to number of vaccine doses and severity of COVID-19 infection and outcomes in terms of mortality and length of stay

| | C 1 | C 2 | C 3 | C 4 | Mortality, n(%) | Mean LOS (days) |
|---|---|---|---|---|---|---|
| Unvaccinated (n = 4) | 0 | 0 | 1 | 3 | 3 (75%) | 13 |
| 1 dose (n = 0) | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 doses (n = 23) | 2 | 0 | 12 | 9 | 5 (21.7%) | 15.5 |
| 3 doses (n = 14) | 0 | 8 | 1 | 5 | 1 (7.1%) | 14 |

LOS – length of stay

The median length of stay was 16 days, ranging from 5 to 57 days. There were 9 (22.0%) patients who passed away in hospital. Table 3 shows the number of patients and COVID-19 vaccine doses received for each severity category, and the mortality rate and length of stay outcomes based on vaccination doses and severity of infections.

**DISCUSSION:**

This was a retrospective audit of COVID-19 patients admitted under Geriatric Medicine to review the compliance with the local COVID-19 management guidelines. A significant proportion of the patients were relatively immobile and dependent on activities of daily living. This was similar to a descriptive study of geriatric medicine patients admitted to the hospital prior to the pandemic [6].

In this audit, just over 80% of patients received intravenous remdesivir, while among the oxygen dependent patients (C4), almost 90% of the COVID-19 patients received dexamethasone. A Spanish retrospective review of patients older than 80 years old hospitalized with COVID-19 infections showed that treatment with remdesivir had a lower 30-day all-cause mortality rate [7].

The RECOVERY Trial randomized patients to receive dexamethasone compared to usual care showed a reduction in 28-day mortality for patients on oxygen at randomization or those receiving mechanical ventilation [8]. In addition, a retrospective case-control study from 36 hospitals in France and Luxembourg showed that for patients aged 80 years and older and required oxygen had a reduced 14-day mortality among those treated with corticosteroids [9]. While the compliance rates for treatment with remdesivir and dexamethasone were both above 80%, this should be improved further given the high risk of complications and mortality for older patients with COVID-19 infections.

The main medication used for venous thromboembolism (VTE) prophylaxis in RIPAS hospital is fondaparinux. A prospective cohort study from the United States showed that hospitalized patients with COVID-19 infections not on anticoagulation therapy had a 2.26 times increased risk of mortality [10]. A meta-analysis showed that incidence of VTE was up to 26% in hospitalized COVID-19 patients [11]. Thus, VTE prevention should be considered for high risk COVID-19 patients, which according to the local guideline is recommended for all oxygen dependent patients. This was only prescribed for about 70% of the patients. While this is a slight improvement from a 50% rate of VTE prophylaxis in a previous audit [12], there remains further room for improvement in this aspect.

Finally, despite the small numbers in the audit, there was an observable association between COVID-19 vaccination doses and reduced mortality. This supports the overall recommendations that older people should receive COVID-19 vaccinations to reduce the risk of severe infections and mortality [13].

## CONCLUSION:

While there appears to be a relatively high rate of compliance with COVID-19 management guidelines in older people admitted to RIPAS Hospital, there is still some room for improvement, given that older people are at high risk of poor outcomes with COVID-19 infections.

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# CROSS-SECTIONAL STUDY OF THE KNOWLEDGE, ATTITUDE, PRACTICES AND CONCERNS (KAPC) ABOUT COVID-19 VACCINATION AMONG STUDENTS IN UNIVERSITY OF PAPUA NEW GUINEA

Running title: Covid-19 Vaccination Hesitancy Among University Students

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**ABSTRACT**:

After the declaration of the COVID-19 pandemic by the WHO in March 2020, the government of Papua New Guinea (PNG) took extraordinary measures to inform and educate the people about the ways of preventing the spread of the deadly virus. Massive awareness campaigns were carried out using most of the available media outlets in the country. The COVID-19 vaccination campaign was launched in March 2021 for healthcare and frontline workers, including medical and health science students. However, many eligible candidates for vaccination were hesitant to accept the vaccine. It was therefore necessary to determine the level of COVID-19 vaccine hesitancy by assessing the students' Knowledge, Attitudes, Practices and Concerns (KAPC) regarding the vaccine. The major aim of this study was to assess the KAPC of students in the University of Papua New Guinea (UPNG), using a questionnaire-based survey. This institutional-based cross-sectional observational descriptive study was carried out in both UPNG campuses, Taurama and Waigani. The study population included both residential and non-residential students. A validated, pretested, self-assessed questionnaire was used to collect information on socio-demographics, social interactions, information-seeking behavior and the KAPC about COVID-19 vaccines. A total of 768 questionnaires were distributed to students. However, based on the inclusion criteria, a total of 118 (15.4%) of the questionnaires collected were excluded. Of the 650 remaining questionnaires 300 were in Taurama and 350 in Waigani. The response rate for Taurama campus was 81.7% compared to 71.1% for the Waigani campus. Knowledge: 98% of students in Taurama, as opposed to 82.3% in Waigani, stated correctly that COVID-19 is caused by a virus. Only 51.8% in Taurama and 31.7% in Waigani said that the genetic material in the virus is RNA. 88.2% in Taurama and 78.3% in Waigani stated that COVID-19 cannot be transmitted by mosquito bites. Knowledge scores for students in Taurama and Waigani were 61.6% and 49.7% respectively. Binary logistic regression analysis showed a significant association between residence and students' Knowledge about the virus and the vaccine. Students in Taurama were about four times more likely to have good knowledge about the virus

and the vaccine than students in Waigani (OR 3.84, 95% CI: 2.64 - 5.58, p-value=<0.001). The low vaccination rate amongst UPNG students was seen as a consequence of their poor knowledge which, in turn, causes poor attitudes, practices, and concerns regarding the efficacy/safety of the vaccine.

**Keywords**: COVID-19, Vaccines, Knowledge, Attitude, Practices, Concerns, Students

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**INTRODUCTION:**

In December 2019, an outbreak of pneumonia cases in Wuhan, China, was later linked to a new virus, 2019-nCoV, so called then [1, 2, 3]. The World Health Organization (WHO) declared 2019-nCoV a Public Health Emergency of International Concern (PHEIC) on 30 January 2020. On the 11 February 2020, the WHO named the disease COVID-19, short for "Coronavirus Disease 2019" [2, 3, 4]. On the 12 March 2020 COVID-19 was declared a pandemic by the WHO [4].

COVID-19 pandemic affected the day-to-day life in multiple ways, causing lockdowns to reduce the rapid spread of the virus [5]. The complexities of modern medicine and research were aggravated by the emerging variants of nCoV-2 which influenced the responses to the drugs and vaccines designed for disease management. These uncertainties and prolonged lockdowns affected people's mental health, causing serious psychological disturbances, such as, depression, anxiety, and inability to tackle negative emotions; these, in turn, led to an increase in suicidal behaviors which became a subject of concern, especially among the youths [6]. Some of the groups that were affected severely include school children, university students, youths, low-income earners, shop-owners, and health professionals.

The COVID-19 Treatment Guidelines Panel (the Panel) recommends COVID-19 vaccination as soon as possible for everyone who is eligible according to the Centers for Disease Control and Prevention's Advisory Committee on Immunization Practices [7].

However, despite immense efforts made to develop safe and effective COVID-19 vaccines, many people including students were hesitant to accept the vaccines [8]. "COVID-19 Vaccine hesitance" can be defined as "low acceptance" or even "rejection" of COVID-19 vaccines by a population, despite the then ongoing vaccination drives. Vaccine hesitancy was listed among the top ten global threats of 2019 [8, 9].

Multiple factors, such as knowledge/perception of the likelihood of the COVID-19 spread, the perceived efficacy and safety of the new vaccines, the logistics of vaccine distribution – all have a bearing on the levels of public acceptance of the vaccine in general.

An assessment of the Knowledge, Attitude, Practices, and Concerns (KAPC) regarding COVID-19 vaccines, therefore, is a logical prerequisite for improving the vaccine acceptance levels among the population, especially in the resource limited countries [10].

Papua New Guinea (PNG) response to the COVID-19 pandemic:

On the 20 March 2020 the Prime Minister of PNG announced the first positive COVID-19 case in the country [11]. On 22 March 2020, a "State of Emergency" (SOE) for 14 days was declared. The entire country was put on a 14-day lockdown. PNG government established the National Emergency Operation Centre (NEOC) which was a multi-ministerial and inter-agency coordination body, to coordinate most of the strategic planning and operations of the health and non-health aspects of government [11]. The SOE was then extended for another two months, to enhance government preparedness measures to respond to COVID-19. The SOE enabled the whole-of-Government approach to the COVID-19 response. PNG Joint Agency Task Force National Control Centre (PNG JATF NCC) for COVID-19 was established under the National Pandemic ACT 2020. On Tuesday, 9 March 2021, it was reported that the PNG Medical and Scientific Advisory Committee (MESAC), after studying the various COVID-19 vaccines available, had recommended that PNG source the AstraZeneca vaccine which was supplied through the COVID-19 Vaccine Global Access (COVAX) facility and approved by the WHO [11, 12]. On Thursday, 3 June 2021, "Directive No. 2" authorized a voluntary AstraZeneca vaccination to be administered in the vaccination roll-out program in PNG [11]. Approval was also given for the administration of a booster dose or second dose which was also optional. The vaccine was made available freely for those over 18 years of age. However, despite the massive awareness campaigns, only a small proportion of eligible candidates for vaccination, even among students, responded to the appeal to be vaccinated. A survey conducted by the World Bank reported that approximately 18% of PNG citizens were planning to get vaccinated, 26% did not want the vaccine, and 55% had not yet decided; 80% of respondents stated that they had concerns about the side effects of the vaccine, while 50% had no trust in the vaccine [13].

There are, however, no published studies yet on the KAPC among university students. Some studies conducted so far among the general

population have assessed public opinion regarding the COVID-19 vaccine before it was introduced in real time [10, 14]. These studies have used semi-structured questionnaires that had not been validated [10, 14]. Furthermore, these survey tools were administered in populations with vastly diverse socio-demographic and cultural backgrounds which influence the acceptance of COVID-19 vaccine. Some authors have emphasized the need to develop a standard questionnaire, certified or approved by the WHO, for a more uniform interpretation of the views of the target populations regarding the COVID-19 vaccines [15]. The assumption is that this will assist public health authorities in developing uniform advocacy and awareness messages to educate the population in the various countries regarding the need for the approved COVID-19 vaccination.

Vaccine hesitancy during the COVID-19 pandemic is caused by misinformation and lack of adequate knowledge about the COVID-19 vaccines. Thus, the need to assess people's KAPC about COVID-19 vaccines in resource limited countries with diverse cultures like PNG cannot be overemphasized.

The objective of the current study was to use an approved and validated questionnaire [16] to assess KAPC about the COVID-19 vaccines among students in the two campuses in the University of PNG (UPNG), in order to identify the barriers to the acceptance of the COVID-19 vaccine.

**METHODOLOGY:**

Study sites and subjects: This study was carried out in UPNG, the premier university in PNG. The university is made up of five schools. Four of the five schools (School of Natural and Physical Sciences, School of Humanities and Social Sciences, School of Business and Public Policy and School of Law) are located in the Waigani campus, while the School of Medicine and Health Sciences is located in the Taurama campus. Students in the Taurama campus complete their foundation year in the Waigani campus before moving over to Taurama to pursue the degree in medicine, dentistry or the health sciences. Post-basic nurses from various hospitals around the country are also enrolled in the Taurama campus to complete the nursing degree. The study subjects include students that are currently registered in both UPNG campuses. The study was conducted from May to August 2022.

Sample size: Currently, there is no published data on the prevalence of COVID-19 among students in the UPNG. To obtain the sample size an equation for computing sample size for unknown population size was used, where; Sample Size = $(\text{Z-score})^2 * \text{StdDev}*(1-\text{StdDev}) / (\text{margin of error})^2$.

Using the equation, a 95% confidence interval level (Z score =1.96), 0.5 standard deviation, and a margin of error (confidence interval) of +/- 5% was chosen.

The calculated sample size was 384 students in Taurama campus and 384 students in Waigani campus. The total sample size of 768 was considered to be adequate for a mini-survey with limited resources.

Study design and sampling: This was an institution-based cross-sectional observational descriptive study. The target population consisted of only registered students, whether residential or non-residential, in both UPNG campuses. All registered students in their second year or higher were eligible to participate in the study. Simple random sampling was used in the selection of survey participants.

Inclusion criteria: The students were informed about the nature of the study and that their participation was entirely voluntary. All consenting students above 18 years of age were eligible to participate in the study.

Data collection using a validated questionnaire: In the present study a reliable and validated questionnaire prepared by Kumari et al [16] was used for data collection. The questionnaire was modified to assess UPNG students' Knowledge, Attitude, Practices, and Concerns (KAPC) about COVID-19 vaccines. The modified questionnaire was pre-tested among 20 randomly selected students. Feedback and suggested changes were provided orally and in writing. The feedback was used to improve the questionnaire which was used in the present study.

The questionnaire contains two sections (A and B). Section-A probes the respondents' socio-demographic profile and acceptance of COVID-19 vaccines. Section-B seeks to elicit information about the respondent's Knowledge (as well as the sources of that knowledge) about COVID-19 and explores their Attitude, Perception and Concerns regarding the COVID-19 vaccines. In the analysis of the respondent's Knowledge, each correct answer was coded as "one", while each incorrect answer or "don't know" was given zero. A 5-point Likert scale was used to gauge the respondents' attitudes, practices and concerns: strongly agree (5 points), agree (4 points), neutral (3 points), disagree (2 points) and strongly disagree (1 point).

Statistical analysis and interpretation of the data**:** Before the statistical analysis, completeness of the data was evaluated. The completed questionnaires were collected, coded and entered into Microsoft Excel Spreadsheets for analysis. Further statistical data analysis was performed using IBM SPSS version 22 (Statistical Package

for the Social Sciences). Descriptive and inferential statistics were used to analyse data, obtained samples, and make inferences about the study population. Descriptive statistics were used to analyse demographic characteristics such as, gender, and residence (campus). Inferential statistics such as Chi-square test were used for analysing categorical variables, such as gender and residence/campus. Chi-square tests were used to compare proportions at significance level $p \leqslant 0.05$, and also to assess the relationship and or association between the independent and dependent variables. T-test (Student's t-test) was used to compare knowledge, attitude, practice and concern scores of students in Taurama and Waigani campuses.

Primary outcome variable (dependent variable) were students' knowledge, attitudes, practices and concern independent variables of interest were gender, and residence. A binary logistic regression model was used to model students' knowledge, attitudes, practices and concerns related to COVID-19.

Interpretation of the KAPC scores: The numbers of correct answers for Q7 to Q12, Q15 to Q18, Q19 and Q20 to Q21 were used to calculate the Knowledge, Attitude, Practices and Concerns scores, respectively. The knowledge score for each participant was determined by allotting a score of '1' to correct responses and '0' (zero) to incorrect and 'don't know' responses.

Attitudes, Practices and Concerns toward COVID-19 vaccine were assessed using the 5-point Likert response scale (1 point for 'strongly disagree' to 5 points for 'strongly agree'). However, for the calculation of the Attitudes, Practices and Concerns scores, the 5-point Likert scale was modified to a 3-point scale. The 3 categories were Agree (strongly agree + agree), Neutral and Disagree (disagree + strongly disagree) [18].

To obtain the score for a section, the number of correct answers obtained was expressed as percentage of the total number of correct answers for the section. The criteria proposed by Hasan et al [23] were used for the categorization of the KAPC levels of the respondents. Score $\geqslant$ 75% was categorized as Good. Scores between 74 – 65% were categorized as Fair. Scores below 64% were categorized as Poor.

Ethical clearance: Ethical approval was obtained from the PNG National Department of Health Medical Research Advisory Committee (NDoH MRAC) and the Ethics and Research Grant committee in School of Medicine and Health Sciences (SMHS), University of Papua New Guinea (UPNG).

Written/Signed informed consent was obtained from each of the participants. The consent was documented on each participant interview form.

This consent procedure was approved by the ethics committees.

**RESULTS:**

Exclusion criteria: The total number of questionnaires distributed was 768. This was made up of 384 in Taurama campus and 384 in Waigani campus. For the exclusion criteria, questionnaires completed by students below 18 years of age were excluded. A total of 84 (21.9%) of the questionnaires from Taurama campus and 34 (8.9%) from Waigani campus were excluded because they were completed by students below 18 years of age.

Demographic characteristics:

Of the 300 questionnaires from Taurama campus, 245 were suitable for analysis (response rate of 81.7%). In Waigani campus, 249 of the 350 questionnaires were suitable for analysis (response rate of 71.1%).

In Taurama campus, 44.5% (109) of the participants were female and 55.5% (136) were male students. In Waigani campus, 53.8% (134) were female and 46.2% (115) were male students. Further detailed analysis of the data was not separated according to gender.

When asked if they had been vaccinated, 39.6% (97) in Taurama and 22.9% (57) in Waigani said they had been vaccinated. Thus, between 60 to 75% of the students in Taurama and Waigani were unvaccinated at the time of this study.

The students were asked if it was legal to take the COVID-19 vaccination in PNG. Only 10.6% (26) and 11.3% (28) in Taurama and Waigani campuses respectively, responded in the affirmative.

**Knowledge (K)**: The responses to the knowledge questions about the vaccine are presented in Table 1. In Taurama, 95.9% (235) of respondents, compared to 88.5% (221) in Waigani, said that people above 18 years of age were eligible {E} for vaccination in PNG. Majority of the respondents in Taurama (83.2%) and Waigani (89.6%) correctly stated that infants below one year of age were not eligible {NE} for the COVID-19 vaccination in PNG. About twice as many respondents in Taurama compared to Waigani (43.7% vs 22.9%) stated that patients with chronic diseases were eligible to take the COVID-19 vaccine in PNG.

The responses to Knowledge questions (Q8 to Q12) about the virus and the vaccine are presented in Table 2. When asked about the causative agent of COVID-19 infection, 98.0% (240) in Taurama and 82.3% (205) in Waigani correctly stated that it was a virus. The follow-up question was about the genetic material of the COVID-19 virus. In response, 51.8% (127) in

Taurama and 31.7% (79) in Waigani stated correctly that the genetic material is RNA. Malaria infection is very high in PNG; thus, the students were asked if COVID-19 can be transmitted by a mosquito bite. In Taurama, 88.2% (216) and 78.3% (195) in Waigani stated that it cannot be transmitted by mosquito bites. In response to Q 12 on how an infected person can spread the infection, 98.8% (242) in Taurama and 92.8% (231) in Waigani gave correct answers.

The next question (Q 13) was whether a second or booster dose of the vaccine was needed to increase the immunity against the virus. In Taurama 55.1% (135) of the students answered in the affirmative, compared to 34.5% (86) in Waigani.

Results obtained when the data for all the Knowledge questions (Q7 to Q 13) were further analyzed are summarized in Table 3.

Table 4 presents students' responses to the question (Q 14) about the sources of information that influenced their decisions and about the relative significance of those sources. In Taurama, 66.9% (164/245) and in Waigani, 59.4% (146/249) stated that "Health care providers – doctors, nurses and others" had a "very significant effect" on their opinion about COVID-19 vaccination. Second in significance came the News from National TV and FM Radio: Taurama 36.7% (90/245) and Waigani 44.6% (111/249).

Table 1: Responses (%) to questions on Knowledge {Taurama: N = 245; Waigani: N = 249}
(K: Knowledge questions)

| Q7 | We have mentioned a group of people who may or may not be eligible for taking COVID-19 vaccine. Please indicate, in each case, the most appropriate option listed for each of the groups (Knowledge about COVID-19 and the vaccine) | | | | | | |
|---|---|---|---|---|---|---|---|
| | | Eligible % | | Not eligible % | | Don't know % | |
| | Group | Taurama | Waigani | Taurama | Waigani | Taurama | Waigani |
| (i) | Infants below one year of age | 3.3 | 1.2 | 83.2 | 89.6 | 13.5 | 9.2 |
| (ii) | People below 18 years old | 28.2 | 27.3 | 47.8 | 53.0 | 24.0 | 19.7 |
| (iii) | People above 18 years of age | 95.9 | 88.8 | 0.40 | 4.4 | 3.7 | 6.8 |
| (iv) | Pregnant and lactating women | 35.9 | 20.5 | 32.3 | 52.6 | 31.8 | 26.9 |
| (v) | Patients with chronic diseases, diabetes, hypertension, and heart disease | 43.7 | 22.9 | 35.1 | 51.0 | 21.2 | 26.1 |
| (vi) | Persons having active COVID-19 infection | 57.1 | 75.1 | 25.7 | 8.4 | 17.2 | 16.5 |
| (vii) | Persons recovered from COVID-19 infection | 69.4 | 60.2 | 13.9 | 15.7 | 16.7 | 24.1 |
| (viii) | Persons allergic to food items or drugs | 28.6 | 14.1 | 31.4 | 49.4 | 40.0 | 36.5 |
| (ix) | Immuno-compromised persons | 45.7 | 17.3 | 26.9 | 38.1 | 27.4 | 44.6 |

Table 2: Responses (%) to Knowledge questions (Q8 to Q12) about the virus and vaccine: (Knowledge continues) {Taurama N = 245; Waigani N = 249}

| | | | Taurama % | Waigani % |
|---|---|---|---|---|
| Q8 | What is the cause of COVID-19 infection? | Bacteria | 0.41 | 5.2 |
| | | Virus | 98.0 | 82.3 |
| | | Not sure | 1.6 | 12.5 |
| Q9 | What is the type of genetic material in COVID-19? | DNA | 13.1 | 9.2 |
| | | RNA | 51.8 | 31.7 |
| | | Not sure | 35.1 | 59.1 |
| Q10 | Are antibiotics effective in treatment of COVID-19? | Yes | 21.2 | 22.5 |
| | | No | 48.6 | 36.1 |
| | | Don't know | 30.2 | 41.4 |
| Q11 | Can COVID-19 be transmitted by mosquito bite? . | Yes | 1.2 | 1.2 |
| | | No | 88.2 | 78.3 |
| | | Don't know | 10.6 | 20.5 |
| Q12 | COVID-19 infected person can spread the infection through droplets from coughing or sneezing and also through contaminated hands and surfaces | Yes | 98.8 | 92.8 |
| | | No | 0 | 2.0 |
| | | Don't know | 1.2 | 5.2 |

Table 3. Summary of results for all the Knowledge questions (Q7-13): Correct and Incorrect responses among students in Taurama and Waigani, respectively

| **Questions of knowledge on COVID19** | **Correct answers** | | | | **Incorrect answers** | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Taurama** | | **Waigani** | | **Taurama** | | **Waigani** | | |
| | **N** | **%** | **N** | **%** | **N** | **%** | **N** | **%** | **P-value** |
| Q7.1 Infants below one year of age (NE) | 204 | 83.3 | 223 | 89.6 | 41 | 16.7 | 26 | 10.4 | 0.041 |
| Q7.2 Children and Adolescents below 18 years of age (NE) | 117 | 47.8 | 132 | 53.0 | 128 | 52.2 | 117 | 47.0 | 0.243 |
| Q7.3 Adults above 18 years of age (E) | 235 | 95.9 | 221 | 88.8 | 10 | 4.1 | 28 | 11.2 | 0.003 |
| Q7.4 Pregnant and lactating women (E) | 88 | 35.9 | 51 | 20.5 | 157 | 64.1 | 198 | 79.5 | <0.001 |
| Q7.5 Patients with chronic diseases like diabetes, hypertension, heart disease (E) | 107 | 43.7 | 57 | 22.9 | 138 | 56.3 | 192 | 77.1 | <0.001 |
| Q7.6 Persons having active COVID-19 infection (NE) | 63 | 25.7 | 21 | 8.4 | 182 | 74.3 | 228 | 91.6 | <0.001 |
| Q7.7 Persons recovered from COVID-19 infection (E) | 170 | 69.4 | 150 | 60.2 | 75 | 30.6 | 99 | 39.8 | 0.033 |
| Q7.8 Persons allergic to food items or drugs (E) | 70 | 28.6 | 35 | 14.1 | 175 | 71.4 | 214 | 85.9 | <0.001 |
| Q7.9 Immuno-compromised persons (E) | 112 | 45.7 | 43 | 17.3 | 133 | 54.3 | 206 | 82.7 | <0.001 |
| Q8. What is the cause of COVID-19? (1) Bacteria; (2) Virus; (9) Not sure | 240 | 98.0 | 205 | 82.3 | 5 | 2.0 | 44 | 17.7 | <0.001 |
| Q9. What is the type of generic material in COVID-19? (1) DNA; (2) RNA; (9) Not sure | 127 | 51.8 | 79 | 31.7 | 118 | 48.2 | 170 | 68.3 | <0.001 |
| Q10. Are antibiotics effective in treatment of COVID-19? (1) Yes; (2) No; (9) Don't know | 119 | 48.6 | 90 | 36.1 | 126 | 51.4 | 159 | 63.9 | 0.005 |
| Q11. Can COVID-19 be transmitted by mosquito bite? (1) Yes; (2) No; (9) Don't | 216 | 88.2 | 195 | 78.3 | 29 | 11.8 | 54 | 21.7 | 0.003 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Q12. COVID-19 infected person can spread the infection through droplets from coughing or sneezing and also through contaminated hands and surfaces. (1) Yes; (2) No; (9) Don't know | 242 | 98.8 | 231 | 92.8 | 3 | 1.2 | 18 | 7.2 | 0.001 |
| Q13. 1 First dose of vaccination. (1) Yes; (2) No; (9) Don't know | 72 | 29.4 | 73 | 29.3 | 173 | 70.6 | 176 | 70.7 | 0.986 |
| Q13. 2 Second dose of vaccination: (1) Yes; (2) No; (9) Don't know | 135 | 55.1 | 86 | 34.5 | 110 | 44.9 | 163 | 65.5 | <0.001 |
| Q13. 3 Fourteen days after first dose of vaccine (1) Yes; (2) No; (9) Don't know | 43 | 17.6 | 22 | 8.8 | 202 | 82.4 | 227 | 91.2 | 0.004 |

Table 4: General assessment concerning influence from both media and interactions.
{Taurama campus N = 245; Waigani campus N = 249}

| Q 14: In the present era there are multiple sources of information regarding a particular issue. How significantly the following sources of information have influenced your opinion about vaccination? | **Insignificant effect %** | | **Somewhat significant effect %** | | **Very significant effect %** | |
|---|---|---|---|---|---|---|
| | **Taurama** | **Waigani** | **Taurama** | **Waigani** | **Taurama** | **Waigani** |
| (i) News from National TV / Radio | 14.3 | 11.6 | 49.0 | 43.8 | 36.7 | 44.6 |
| (ii) Government agencies | 17.1 | 17.3 | 53.5 | 53.4 | 29.4 | 29.3 |
| (iii) Social media (Facebook, Instagram, Whatsapp) | 29.4 | 26.5 | 35.1 | 37.8 | 35.5 | 35.7 |
| (iv) Discussion among friends and family | 28.2 | 17.3 | 51.8 | 49.4 | 20.0 | 33.3 |
| (v) Health care providers (Doctors, Nurses, others) | 5.7 | 6.8 | 27.3 | 33.7 | 66.9 | 59.4 |

**Attitude (A):** Four statements (Q15 to Q18) were used to assess the attitude of the students towards the COVID-19 vaccine. The 5-point Likert Scale was used to interpret the responses (Table 5).

In response to Q15 about their willingness to take the vaccine, 21.2% (52/245) in Taurama compared to 10.4% (26/249) in Waigani said that they strongly agreed to take the vaccine. In response to Q16 about their preferred way of acquiring immunity to COVID, 19.6% (48/245) and 24.9% (62/249) in Taurama and Waigani, respectively, strongly agreed that acquiring immunity against COVID-19 naturally, by getting sick, was preferable to vaccination.

In Q17, the students were asked if they would still take the vaccine, even if they had to pay for it. Only 8.2% (20/245) and 2.8% (7/249) in Taurama and Waigani, respectively, said that they strongly agreed. In response to Q18 about whether they would recommend the vaccine to members of their families and friends, 22.9% (56/245) of students in Taurama strongly agreed, compared to only 6.4% (16/249) of their counterparts in Waigani. The detailed results are presented in Table 5.

Table 5: Attitude (A) towards COVID-19 vaccination: Q 15 to Q 18: {T = Taurama N = 245; W = Waigani N = 249)

| In the next 4 questions there are certain statements regarding different aspects of COVID-19 vaccination. Please "tick" the response which best explains your opinion regarding a particular statement (Attitude towards the vaccine): | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Strongly agree %** | | **Agree %** | | **Neither agree nor disagree %** | | **Disagree%** | | **Strongly disagree %** | |
| | **T** | **W** | **T** | **W** | **T** | **W** | **T** | **W** | **T** | **W** |
| Q 15: When it is (was) my turn for vaccination I am (was) willing to take the COVID-19 vaccine. | 21.2 | 10.4 | 22.0 | 15.7 | 17.6 | 20.9 | 20.8 | 20.1 | 18.4 | 32.9 |
| Q 16: I will prefer to acquire immunity against COVID-19 naturally (by having the disease / subclinical infection) rather than by vaccination. | 19.6 | 24.9 | 20.8 | 26.9 | 25.3 | 25.7 | 22.0 | 15.7 | 12.2 | 6.8 |
| Q 17: I am (was) willing to get the COVID-19 vaccine, even if I have/had to pay for it. | 8.2 | 2.8 | 17.3 | 10.0 | 20.0 | 17.7 | 28.2 | 30.9 | 26.1 | 38.6 |
| Q 18: I will recommend my family and friends to get vaccinated against COVID-19 | 22.9 | 6.4 | 20.8 | 16.1 | 30.6 | 30.5 | 14.7 | 21.3 | 11.0 | 25.7 |

**Practices (P)**: Q19 consists of 10 statements (I to X) that were used to assess the Practices of the students towards the vaccine.

The 5-point Likert Scale was used to interpret the responses (Table 6).

In Taurama 15.5% (38/245) strongly agreed that there is no harm in taking the vaccine compared to 6.4% (16/249) in Waigani. In response to the next statement that the vaccine can provide protection against the infection, 24.1% (59/245) and 8.8% (22/249) in Taurama and Waigani respectively said that they strongly agreed. In Taurama, 21.2% (52/245) compared to 7.2% (18/249) in Waigani strongly agreed that the benefits of taking the vaccine outweighed the risks. Only 11.4% (28/245) in Taurama and 6.8% (17/249) in Waigani strongly agreed with the statement that "*there is sufficient data regarding the vaccine's safety and efficacy released by the government*".

In Taurama 13.4% (33/245) compared to 8.0% (20/249) in Waigani strongly agreed with the statement "*My role models / political leaders / senior doctors / scientists have taken the vaccine*".

Table 6: Practice (P) section of the questionnaire:

Q19: If you have taken the vaccine certain factors may have motivated you to do so. If you are waiting for your turn to get vaccinated, then certain factors might be responsible for your decision to take the vaccine. There are certain statements regarding this below. Please "Tick" the response that best explains your opinion for each statement. Please put a tick against each of the statements.
(**Practices regarding COVID-19 vaccine**) {**T: Taurama N = 245; W: Waigani N = 249**}

**I have taken / will take the COVID-19 vaccine because**:

| | Strongly agree % | | Agree % | | Neither agree nor disagree % | | Disagree % | | Strongly disagree % | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **T** | **W** | **T** | **W** | **T** | **W** | **T** | **W** | **T** | **W** |
| (i) I think there is no harm in taking it | 15.5 | 6.4 | 22.5 | 17.7 | 27.3 | 28.5 | 22.5 | 27.3 | 12.2 | 20.1 |
| (ii) I believe COVID-19 vaccine is useful to protect me against the infection. | 24.1 | 8.8 | 36.7 | 25.3 | 22.9 | 34.9 | 9.4 | 18.1 | 6.9 | 12.9 |
| (iii) COVID-19 vaccine is available for free. | 36.7 | 23.7 | 40.8 | 43.8 | 13.5 | 13.3 | 5.7 | 10.0 | 3.3 | 9.2 |
| (iv) My health care professional (doctor / nurse/ pharmacist) has recommended it to me. | 19.6 | 11.2 | 43.7 | 32.1 | 20.8 | 25.7 | 11.4 | 18.9 | 4.5 | 12.1 |
| (v) I feel the benefit of taking the COVID-19 vaccine outweighs the risk involved. | 21.2 | 7.2 | 29.4 | 25.7 | 28.6 | 29.7 | 13.5 | 22.5 | 7.3 | 14.9 |
| (vi) I believe that taking the COVID-19 vaccine is a societal responsibility | 16.7 | 6.8 | 29.0 | 28.9 | 26.5 | 29.7 | 18.8 | 18.1 | 9.0 | 16.5 |
| (vii) There is sufficient data regarding the vaccine's safety and efficacy released by the government. | 11.4 | 6.8 | 24.1 | 22.1 | 23.7 | 22.1 | 24.9 | 29.3 | 15.9 | 19.7 |
| (viii) Many people are taking the COVID-19 vaccine. | 5.3 | 4.0 | 24.5 | 29.7 | 33.1 | 23.7 | 26.1 | 31.7 | 11.0 | 10.8 |
| (ix) I think the vaccine will help in eradicating COVID-19 infection. | 10.6 | 5.6 | 31.8 | 19.7 | 30.2 | 42.2 | 17.1 | 19.6 | 10.2 | 12.9 |
| (x) My role models / political leaders / senior doctors / scientists have taken the vaccine | 13.4 | 8.0 | 33.5 | 34.9 | 27.8 | 27.3 | 16.3 | 18.1 | 9.0 | 11.7 |

**Concerns (C**): Q 20, consisting of 7 statements (I to VI), and Q21 were used to assess students' Concerns regarding the vaccine. The 5-point Likert Scale was used to interpret the responses (Table 7).

23.7% (58/245) of respondents In Taurama and 35.3% (88/249) in Waigani strongly agreed that they believed they "*might have immediate serious side effects after taking COVID-19 vaccine*".

In response to the next statement, that "*COVID-19 vaccine was rapidly developed and approved* ", 28.2% (69/245) in Taurama and 22.5% (56/249) in Waigani said that they strongly agreed.

Another concern that was strongly agreed with by 13.9% (34/245) in Taurama and 15.7% (39/249) in Waigani was that the "*COVID-19 vaccine is being promoted for commercial gains by pharmaceutical companies*".

27.4% (67/245) of students in Taurama, as opposed to 41.4% (103/249) in Waigani, strongly agreed that "*Because of limited awareness done on COVID-19 vaccines to inform people properly, they are having fears of taking the vaccine*

*because they are not really sure whether the vaccine will protect them or not* '

Q21 relates to concerns about the need for precautionary measures after taking the vaccine: "*After getting COVID-19 vaccine, I don't need to follow preventive measures wearing of mask, sanitation and social distancing*". In response, only 7.3% (18/245) in Taurama compared to 12.9% (32/249) in Waigani said that they strongly agree.

Table 7: The Concerns (C) section of the questionnaire.

Q20. There are still several concerns regarding the COVID-19 vaccine that may influence your decision (creating doubt in your mind) to get the COVID-19 vaccine. Give your opinion on how the following statements have influenced / will influence your decision to take the vaccine: (**Concerns about COVID19 vaccine**) {**T: Taurama** N = 245; **W: Waigani** N = 249}

| **I am concerned that**: | Strongly agree % | | Agree % | | Neither agree nor disagree % | | Disagree % | | Strongly disagree % | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **T** | **W** | **T** | **W** | **T** | **W** | **T** | **Wi** | **T** | **W** |
| (i) The vaccine might not be easily available to me | 4.5 | 6.8 | 18.4 | 17.3 | 24.9 | 29.3 | 40.8 | 33.7 | 11.4 | 12.9 |
| (ii) I might have immediate serious side effects after taking COVID-19 vaccine. | 23.7 | 35.3 | 38.4 | 36.6 | 22.0 | 16.9 | 11.0 | 6.0 | 4.9 | 5.2 |
| (iii) COVID-19 vaccine may be faulty or fake | 12.3 | 21.3 | 31.4 | 37.4 | 31.4 | 26.9 | 16.7 | 9.2 | 8.2 | 5.2 |
| (iv) COVID-19 vaccine was rapidly developed and approved. | 28.2 | 22.5 | 43.7 | 38.2 | 19.2 | 26.5 | 5.3 | 5.2 | 3.6 | 7.6 |
| (v) I might have some unforeseen future effects of the COVID-19 vaccine | 26.9 | 33.7 | 39.2 | 44.6 | 23.3 | 13.7 | 6.1 | 3.2 | 4.5 | 4.8 |
| (vi) COVID-19 vaccine is being promoted for commercial gains by pharmaceutical companies | 13.9 | 15.7 | 25.7 | 24.1 | 40.8 | 48.6 | 12.7 | 5.2 | 6.9 | 6.4 |
| (vii) Because of limited awareness done on Covid-19 vaccines to inform people properly, I am having fears of taking the vaccine because I am not really sure whether the vaccine will protect me or not | 27.4 | 41.4 | 34.3 | 33.7 | 16.7 | 11.7 | 15.9 | 8.0 | 5.7 | 5.2 |
| **Q21**. After getting COVID-19 vaccine, I don't need to follow preventive measures wearing of mask, sanitation and social distancing | 7.3 | 12.9 | 8.6 | 9.6 | 14.7 | 26.1 | 34.7 | 27.7 | 34.7 | 23.7 |

Interpretation of KAPC scores:

The Knowledge scores for Taurama and Waigani were 61.6% and 49.7%, respectively, which indicated Poor knowledge about the COVID-19 vaccine. There was a statistically significant difference ($p < 0.05$) between the knowledge scores of students in Taurama and Waigani.

A two-sample t-test was performed to compare knowledge scores of students in Taurama and Waigani. There was a significant difference between knowledge scores of students in Taurama (M=9.6, SD=2.6) and Waigani (M=7.68, SD=2.33); t (492) = 8.690, p=0.008.

A chi-square test of independence showed that there was a statistically significant association between knowledge level (Good/Poor) and students in Taurama and Waigani, $\chi^2$=51.837, df=1, p=<0.001).

A chi-square test of independence showed that there was no significant relationship between knowledge level of students in Taurama and Waigani, and gender (male/female) $\chi^2$=1.815, df=1, p=0.18).

The Attitude score for Taurama was 36.7%, compared to 21.2% for Waigani. Both results indicated Poor attitude towards COVID-19 vaccination. The difference in attitude scores was statistically significant (p<0.05). A chi-square test of independence showed that there was a statistically significant relationship between the attitude towards the vaccine by students in Taurama and Waigani, $\chi^2$=19.36, df=2, p=<0.001). About 60.6% (129) in Waigani disagree compared to 39.4% (84) in Taurama, suggesting a significant difference the students in both campuses have regarding their attitude towards the vaccine. There was no significant association between attitude level (Good/Poor) and gender (p=>0.05)

The Practice score for Taurama was 49.8%, compared to Practice score of 37.8% for Waigani. The difference was statistically significant (p<0.05). The results indicated Poor practices which implies high risk for COVID-19 among respondents in both groups. A chi-square test of independence showed that there was a statistically significant relationship between the practice and students in Taurama and Waigani, $\chi^2$=22.59, df=2, p=<0.001). About 43.7% in Taurama agree that they will take the COVID-19 vaccine compared to 26.9% in Waigani. There was no significant association between Practice level (Good/Poor) and gender (p=>0.05)

The Concern scores were 39.3% and 38.8% for Taurama and Waigani, respectively. There was no statistically significant difference between concern scores for Taurama and Waigani.

Factor(s) associated with Knowledge, Attitude, Practice of UPNG students:

A binary logistic regression was used to assess the strength of a relationship between one dependent variable and independent variable(s). The dependent variables (knowledge, attitude and practice) and independent variable (residence / campus) were modelled. A univariate analysis was perform using one independent variable

(residence / campus) and each of the dependent variable to assess how the factor (residence / campus) influence students' knowledge, attitude and practice. Student's residence may influence on whether students have a good or poor knowledge, attitude or practice towards COVID-19 vaccination. The results are presented in Table 8.

Table 8. Binary logistic regression analysis of COVID-19, Knowledge, Attitude, Practice of UPNG students

| | Knowledge | | | Attitude | | | Practice | | |
|---|---|---|---|---|---|---|---|---|---|
| Variables | OR | 95% CI | p-value | OR | 95%CI | p-value | OR | 95%CI | p-value |
| Residence/Campus | | | | | | | | | |
| 1 Taurama | 3.84 | 2.64-5.58 | <0.001 | 2.14 | 1.49-3.07 | <0.001 | 2.37 | 1.49-3.07 | <0.001 |
| 2 Waigani | 1 | | | 1 | | | 1 | | |

1=Reference group/category

**Knowledge**: Knowledge levels were classified as 1=Good knowledge, 0=Poor knowledge. A binary logistic regression analysis showed that there was a significant association between the residence / campus (1=Taurama, 2=Waigani) and the students' Knowledge about the virus and the vaccine. Students in Taurama were almost four times more likely to have good knowledge about the virus and the vaccine than students in Waigani (OR 3.84, 95% CI: 2.64 - 5.58, p-value=<0.001).

**Attitude**: Attitude level was classified as 1=Good attitude, 0=Poor attitude. In the binary logistic regression analysis, residence/campus was associated with attitude towards COVID-19 vaccination. Students who reside in Taurama campus were twice more likely to have good attitude towards COVID-19 vaccination than the students in Taurama (OR 2.14; 95% CI: 1.49 -3.07, p-value=<0.001).

**Practice**: Practice level were classified as 1=Good practice, 0=Poor practice. In the binary logistic regression analysis, residence/campus was associated with practices of students towards the vaccine. Students who reside in Taurama campus were two times more likely to have good practices towards the vaccine than students in Waigani (OR 2.37; 95% CI: 1.65 -3.41, p-value=<0.001).

## DISCUSSION:

One of the recommended strategies for the control and elimination of an infectious disease, such as COVID-19, is the use of an approved vaccine for mass vaccination of the affected population. This strategy was implemented in PNG after the WHO declaration of the COVID-19 pandemic. The focus of this study was to assess the KAPC of students in Taurama and Waigani campuses in UPNG

regarding the COVID-19 vaccination. This is because the students represent the community and have the potential to greatly influence the response of their parents, relatives, peers and friends towards the acceptance or rejection of the COVID-19 vaccine.

The response rates of students in the Taurama and Waigani campuses were 81.7% and 71.1% respectively. The low response rates on both campuses reflect the problem of doing research requiring voluntary participation. They also indicate the negative attitudes of students, especially of those in Waigani, to issues related to COVID-19. The 81.7% response rate on the Taurama campus was higher than the 78% response rate in a similar study by Raja et al. [19] in Sudan, but lower than the 100% response rate reported by Mose et al. [20] for students in Ethiopia.

At the time of this study, 39.6% of students in Taurama and 22.9% in Waigani had been vaccinated. The low vaccination rate may be due to the lack of knowledge about vaccination, as it is the main cause of vaccine hesitancy. The students in Taurama were 1.35 times more likely to take the vaccine, compared to students in the Waigani campus. Vaccine hesitancy is defined as a delay in the acceptance or the refusal of vaccination even when the vaccine is available. According to some recent studies [19, 20, 21], vaccine hesitancy is one of the biggest global health risks, which existed even before the COVID-19 pandemic. The percentage of vaccine-hesitant students in Taurama (60.4%), unlike that in Waigani, was within the ranges (10.6 to 65.1%) reported for medical and health sciences students in Egypt, Uganda, India and Italy [19, 20, 21].

The government of PNG gave the regulatory approval needed to bring COVID-19 vaccines into the country in March 2021, resulting in the legalization of the COVID-19 vaccine [12, 17]. The very high percentages of students in Taurama and Waigani campuses (89.4% vs 88.7%) who did not know about the legality of the COVID-19 vaccines in PNG indicated students' lack of knowledge and interest in government efforts to mitigate the spread of the virus. Thus, lack of relevant information may be responsible for the poor response about the legality of the vaccination by students in both campuses. Most people, including students, were more concerned about the impact of the lockdown and its effect on their livelihoods than they were appreciative of the concerted efforts of the authorities to reduce the spread of the virus.

Students in Taurama were more knowledgeable than students in Waigani. The Knowledge scores for Taurama and Waigani were 61.6% and 49.7%, respectively, with an odds ratio of 3.84 (95% CI

2.64 – 5.58; p<0.001). One explanation is that the Taurama students study medical and health sciences which inform them of many diseases, including COVID-19. The 61.6% score obtained for students in Taurama was lower than the 78.6%, 72% and 70.2% obtained for medical students in Ethiopia [22], United Arab Emirate [23] and Egypt [24], but higher than the 40.8% to 57.0% scores reported by other authors respectively [22, 25, 26, 27]. Our result highlights the importance of knowledge about COVID-19 as well as that of community awareness regarding the available COVID-19 vaccines, because what people think, based on what they know, drives their behaviour.

Majority of the students in Taurama and Waigani campuses stated that their opinion about COVID-19 vaccines was greatly influenced by "Health care providers – doctors, nurses and others". This is contrary to the general assumption that social media significantly influence the opinion of students [28]. Our findings support the report by Venkatesan et al [21], which states that social media was not found to be significantly associated with vaccine hesitancy among medical students.

In the present study, students' attitude was assessed mainly based on their willingness to take the vaccine and to recommend the vaccine to family members. In Taurama, 21.2% of students, compared to 10.4% in Waigani, said that they strongly agreed with the need to take the vaccine. In addition, 22.9% of students in Taurama, compared with 6.4% in Waigani, strongly agreed that they would recommend the vaccine to members of their families and friends. The significant difference in attitude among the Taurama students might be, as has already been stated, due to better knowledge and awareness of vaccine-preventable diseases like COVID-19. These results are similar to those obtained for students in Uganda [28], India and Jordan [21, 27].

The Attitude score for students in Taurama was 36.7% compared to 21.2% for students in Waigani. These scores are lower than the 84.5%, 78.0% and 72.0% attitude scores reported for students in Uganda, Bangladesh and Jordan respectively [22, 24, 25]. The result suggests the need for effective awareness campaigns, to provide the students with a better understanding of the importance of vaccination against infectious diseases like COVID-19.

In the present study, Practices regarding the vaccine were assessed based on the responses to specific questions using the modified 3-point Likert scale. In Taurama 38.0% of students and 24.1% in Waigani agreed that there was no harm in taking the vaccine. That the vaccine is useful and can provide protection against the infection, was agreed upon by 60.8% of Taurama students,

compared with 34.1% in Waigani; odds ratio 3.37. In Taurama, 50.6% of students compared to 32.9% in Waigani, agreed that the benefits of taking the vaccine outweigh the risks involved. The statement that, "sufficient data regarding the vaccine safety and efficacy was released by the government" was accepted by 35.5% of students in Taurama and 28.9% in Waigani. Only 42.4% of student in Taurama and 25.3% in Waigani agreed that the vaccine will help in eradicating COVID-19 infection. The Practice score for Taurama was 49.8% compared to 37.8% for Waigani. The results indicate the knowledge about COVID-19 gap between the medical and health science students in Taurama campus, and students in the Waigani campus. The Taurama students' practice score with regard to COVID-19 vaccines was higher than the 41.0% obtained for students in Uganda but similar to the results (49.2%) for students in Egypt [20, 22, 25, 29].

Some of the responses to the practice questions by students in both campuses might have been influenced by the widespread misinformation about the side effects and ineffectiveness of the COVID-19 vaccines.

Students' Concerns regarding COVID-19 vaccines were also assessed, using the 3-point modified Likert Scale. Majority of the students in Taurama (62.1%) and Waigani (71.9%) believe that they might have immediate serious side effects after taking the COVID-19 vaccine. Similarly, 71.9% of students in Taurama and 60.7% in Waigani expressed the fear that COVID-19 vaccines had been developed too rapidly. Another concern expressed by 39.6% in Taurama and 39.8% in Waigani was that the COVID-19 vaccines are promoted for commercial gains by pharmaceutical companies. 61.7% of students in Taurama and 75.1% of students in Waigani indicated that their fear of taking the vaccine was mainly caused by not having been properly informed about COVID-19 vaccines; they were really not sure that the vaccines can protect them from getting sick. The calculated concern scores for Taurama and Waigani were 39.3%, and 38.8%, respectively.

Unlike reports by some studies [21, 28], the lack of trust in public health experts was not one of the concerns expressed by students in our study. However, there was a very weak correlation between trust in public health experts and willingness to accept the COVID-19 vaccine by students in Taurama and Waigani campuses (rho = 0.01, $p > 0.05$).

These results indicate that Poor Knowledge is one of the main causes of vaccine hesitancy among students on both campuses. It is also directly related to the poor attitudes and practices on the part of the students, as well as to their concerns.

Some of the reasons for low vaccination rates, identified in this study, include lack of knowledge about COVID-19 and the vaccines against it, concerns about the possibility of serious side effects, uncertainty about the efficacy of the available vaccines, misinformation, and insufficient information about the safety of the vaccines. Our results indicate that the major barrier to UPNG students' acceptance of the COVID-19 vaccine is their insufficient knowledge about the safety and efficacy of the vaccine.

The limitations of the study:
The low response rate among students in both campuses and the use of the self-assessed questionnaire can be considered as the limitations of this study.

**CONCLUSION:**

Vaccination is one of the promising strategies for the control and elimination of the COVID-19 pandemic. Vaccine hesitancy is influenced by multiple factors. The response of the university students to the COVID-19 vaccination campaign in PNG was poor. At the time of this study, only 39.6% of students in Taurama and 22.9% in Waigani had received the first dose of the vaccine.

The Knowledge scores for students in Taurama and Waigani were 61.6% and 49.7%, respectively. The Attitude score for Taurama students was 36.7%, compared to 21.2% for students in Waigani.

The Practice score for Taurama was 49.8%, compared with 37.8% for Waigani. These results indicate the knowledge gap about COVID-19 between the two groups of students. The barriers causing the low vaccination rates in this study include poor knowledge, which is directly related to poor attitudes and practices; concerns about serious side effects, lack of trust in the efficacy of the vaccines, misinformation, and insufficient information about vaccine safety.

There is a need for effective awareness campaigns, especially in the Waigani campus, to improve the students' knowledge about the COVID-19 virus and the role of vaccines in combating it.

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# COVID-19, PNEUMOCOCCAL AND INFLUENZA VACCINATIONS AMONG GERIATRIC MEDICINE OUTPATIENTS IN BRUNEI DARUSSALAM

Running Title: COVID-19 pneumococcal flu vaccines in elderly

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**ABSTRACT**:
Vaccines are an important public health measure against infectious diseases. Older people are vulnerable to complications from infections. COVID-19 vaccines, pneumococcal and influenza vaccines are recommended for older people. The electronic records of Geriatric Medicine Outpatients in RIPAS Hospital for August 2022 were reviewed to identify the uptake of these vaccines. Among 49 patients, 61.2% should have been recommended an additional COVID-19 vaccine dose as they were aged 80 years or older, while a further 18 (36.7%) should also be considered due to comorbidities. Only one patient received a pneumococcal vaccine, while none of the patients received annual influenza vaccines. Although there was a high national vaccination rate, further work is required to encourage older people to receive the booster doses, as well as pneumococcal and influenza vaccines. Outpatient clinics should be viewed as an opportunity to counsel patients to get vaccinated.

**Keywords**: Aged, COVID-19, Influenza, Pneumococcus, Vaccination.

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## INTRODUCTION:

Vaccination programmes are important public health measures that enable containment of the spread of various infectious diseases. High immunization rates have resulted in eradication of several viral and infectious diseases [1], and is a key strategy in managing the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections that caused the global coronavirus disease 2019 (COVID-19) pandemic.

Older people are more prone to infections, and old age is a significant risk factor for severe illness [2].

During the COVID-19 pandemic, older people were a vulnerable group, with a higher risk for severe infections, complications and death. It is hypothesised that immune-senescence plays a role in augmenting their susceptibility towards severe illness [3]. For people aged 76 years and older, the COVID-19 estimated mortality rate is 18% [4]. Thus, older people were a priority group in most countries for COVID-19 vaccination programmes.

Brunei has a high COVID-19 vaccination rate, with 99.32% of the population receiving at least two doses (as of 30th December 2022) [5]. Older people were prioritised since the roll-out of the COVID-19 national immunization programme on 3rd April 2021. A third COVID-19 vaccine dose or booster was recommended for people aged 60 years and older since 26th November 2021. In addition, for people aged 80 years and older, and older people with comorbidities, a fourth COVID-19 vaccine dose was recommended since 15th June 2022 [6].

The latter recommendation was supported by a Singapore study, showing that four doses of mRNA vaccines (BNT162b2 or mRNA-1273) significantly reduced the risk of symptomatic SARS-CoV-2 infection, hospitalization and severe disease among people aged 80 years or older compared to three-doses only [7].

Older adults are also recommended to have the pneumococcal vaccine and an annual influenza vaccine [6]. During the pandemic, these additional vaccine recommendations may have taken a backseat, given the spotlight on COVID-19 immunisations.

However, given the rebound of other respiratory illnesses, such as influenza and respiratory syncytial viruses during the post-pandemic period, there is a renewed interest in re-emphasizing these other vaccines in older people [8].

In this paper, the vaccination rate of COVID-19, pneumococcal and influenza vaccines in older people in a Geriatric Medicine Outpatient clinic based in Raja Isteri Pengiran Anak Saleha (RIPAS) hospital was described.

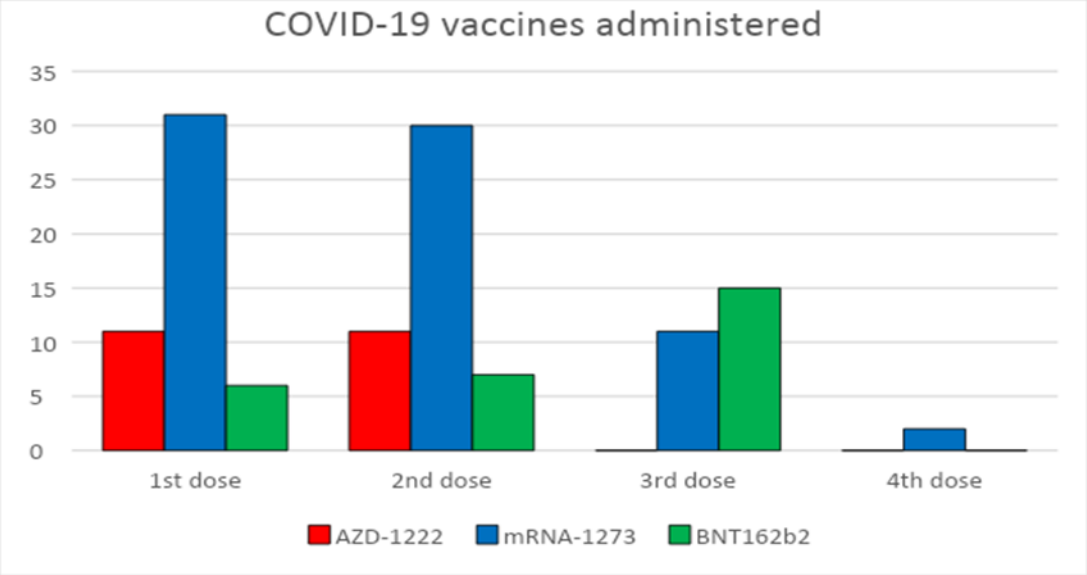
**METHODOLOGY:**

This was a retrospective review of the electronic records of patients attending geriatric medicine outpatient clinics in RIPAS Hospital from 1st August 2022 to 31st August 2022. Demographic information including age, gender, mobility, type of COVID-19 vaccine received, pneumococcal vaccine and influenza vaccine status were collected. It was also assessed whether patients were advised to receive vaccines according to local guidelines during the clinical consultation.

**RESULTS:**

There were 49 patients with a median age of 82 (range 70-102) years. There were 20 (40.8%) males and 29 (59.2%) females. The majority were mobilizing independently (17,34.6%) or walking with a stick (15,30.6%), while 5 (10.2%) used a frame, 10 (20.4%) were limited to wheelchair transfer and two were immobile. Among those who received COVID-19 vaccines, 22 (44.9%) had two doses, 24 (49.0%) had 3 doses and two patients received four doses. One patient was unvaccinated.

Figure 1 shows the type of COVID-19 vaccine administered, which were as follows: Astra-Zeneca 22 (17.7%), Moderna 74 (59.7%) and Pfizer 28 (22.6%).




There were 30 (61.2%) patients who should have been recommended an additional COVID-19 vaccine dose as they were aged 80 years or older; while among younger age groups, a further 18 (36.7%) patients should consider an additional booster dose due to comorbidities. Only 4 (8.3%) out of the 48 patients were counselled regarding this. In terms of other recommended vaccinations, only one patient received a pneumococcal vaccine in 2019, while only 9 (18.4%) patients have ever received an influenza vaccine.

There were no patients who received annual influenza vaccines, while only one clinic patient was counselled to get it.

**DISCUSSION:**

The vaccination rate of with COVID-19 vaccines, pneumococcal and influenza vaccines among

older people attending Geriatric Medicine outpatient clinics was reviewed. Although the national COVID-19 immunisation rate was quite high, we found a significant number of patients who should receive additional COVID-19 vaccine doses according to the national recommendations. As older people are especially vulnerable to complications from COVID-19 infections, discussions to encourage the uptake of COVID-19 vaccine booster doses should be considered as part of a routine clinical consultation.

In addition, there was a minimal uptake of pneumococcal and influenza vaccines among these patients. Given the increased global risk of rebound influenza outbreaks [9], the efficacy and safety of these vaccines, as well as the risk of complications from pneumococcal and influenza infections in older people [10], further effort is also required to promote older people to receive these vaccines. Outpatient clinics should be viewed as an opportunity to counsel patients to get vaccinated.

Further effort is required to improve health professional awareness regarding encouraging patients to receive vaccines, with further studies required to identify the reasons for reduced vaccine uptake among older patients.

**CONCLUSION:**

There is a need to improve the uptake of booster doses of COVID-19 vaccines, pneumococcal and influenza vaccines in older people attending Geriatric Medicine clinics.

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# *MOMORDICAL CHARANTIA* AND *OCIMUM GRATISSIMUM* REVERSE SCOPOLAMINE- OR HIGH-FAT DIET-INDUCED SPATIAL MEMORY IMPAIRMENT BY REGULATING CHOLINERGIC SYSTEM AND OXIDATIVE STRESS IN RATS

Running Title: Memory Restorative Role of Medicinal Plants

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**ABSTRACT**:
This study aimed to explore the effect of *Momordical charantia* (MC), *Ocimum gratissimum* (OG) alone or their synergistic action on memory impairment induced either by scopolamine treatment or high fat diet in rats. Using Morris water maze (MWM) test, we show that treatment of adult rats with scopolamine or high fat diet (HFD) caused a significant impairment in spatial memory. Specifically, the time to locate the hidden platform in MWM (a memory index) was significantly higher in scopolamine- and HFD- treated rats. However, treatment with MC or OG prevents the cognitive impairment induced by the two animal models of Alzhemier's disease (AD). To understand the mechanisms of actions of MC and OG, we examined the brain Acetylcholinesterase (AchE), Malondialdehyde (MDA) and Glutathione (GSH) activities in the brain as well as plasma cholesterol level. We found that scopolamine or HFD treatment significantly increased their activities in the brain and increase plasma cholesterol level in HFD-treated rats. Treatment with MC or OG restores these activities. In conclusion, our findings indicate that the treatment with MC/OG alone could improve the memory function in animal model of AD by regulating the brain cholinergic system and oxidative stress as well as plasma cholesterol level.

**Keywords**: *Momordica charantia*, *Ocimum gratissimum*, Alzheimer's disease, scopolamine, high fat diet.
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**INTRODUCTION**:

Dementia is a form of neurodegenerative disorder that is characterized by decline in the ability to think and remember events which negatively affects the person's daily functioning [1]. Apart from cognitive deficit, other symptoms of dementia are decreased motivation, emotional problem, language difficulties among others [2]. Increase in age is most important risk factor but it is not part of the normal aging process [3]. However, research has revealed a strong correlation between unhealthy life styles (obesity, physical inactivity, intake of alcohol, smoking diabetes) and early onset of dementia [4,5]. The most common type of dementia is Alzheimer's disease (AD) that account for 60 to 70% of cases [3]. Other types are vascular dementia (25% of cases), Lewy bodies (15% of cases) [2]. Less common types are Parkinson disease dementia, mix dementia among others [6,7].

Globally, over 54 million people have been reported to have dementia, 60% of these live in low and middle income countries, with an annual 10 million new cases [8]. It has been projected that more than 75 million people will have dementia globally by the end of year 2030 and this figure will rise to 139 million by 2050. This significant increase is being attributable to rise in the number of people with dementia living in low and middle income countries [9].

Dementia places a great burden on the families and caregivers physically, socially, psychologically and financially. World-wide, the cost of caring for people with dementia as at 2019 was over 1.1 trillion US dollars which is expected to double by 2030 [8]. Dementia is currently being managed by medications to treat its cognitive problem, some of which are acetylcholine esterase inhibitors (Donepezil or Galantamine). However, the use of these drugs in mild cognitive impairment has not shown a significant benefit. In addition, most often these drugs are associated with side effects like bradycardia, decrease appetite and others. Therefore, there is need to explore options in traditional medicine system. Similarly, the World Health Organization (WHO) encourages scientific research into indigenous herbal medicine [10]. About 80% of the world population depends mainly on medicinal plants for their primary health care [11] and the remaining 20% in developed countries like Europe still make use of products derived from plants for their health care system; for instance plant derived alkaloid (galantamine) gotten from Galanthus nivalis is used in the management of neurodegenerative disorders.

Ocimum gratissimum belongs to the Lamiaceae family, it is known as African basil and its one of the best known specie [12]. It is native to Africa and Southern Asia. It is used in food as condiment and also used widely in traditional medicine such as Ayurveda, Chinese traditional medicine and other folk medicine for treating digestive system disorders, infections,

whooping cough and various types of fever [12]. Studies have reported anti-inflammatory, antinociceptive, antipyretic, gastroprotective, hepatoprotective and antimutagenic effects of O. gratissimum [13-19].

Momordical charantia is a flowering plant that belongs to the Cucurbitanceae family. It originated from Africa but it is now cultivated mostly in Asia and the Caribbeans [20]. It is known as bitter melon in English, Ejirin in Yoruba (Nigeria). It has a bitter taste which is more pronounced when it is ripe. The plant has been used in traditional and folk medicine [21] for the treatment of cancer, diabetes, hypertension, hyperlipidemia, and inflammation, viral and bacterial infections [22]. The plant is rich in phytochemicals with many health promoting effects such as terpenoids, proteins, saponins, flavonoids, phenols, essential oil, glucose [23, 24].

In the present study, we explore the potential effects of MC and OG in the management of scopolamine- or high fat diet-induced cognitive impairment in adult rats.

## MATERIALS AND METHODS:

Plant materials and authentication:

Fresh leaves of both *Momordica charantia* and *Ocimmum gratissimum* were purchased from Oro, Kwara state, Nigeria. The leaves were authenticated in the Department of plant Biology, University of Ilorin, Nigeria. Fresh leaves of the two plants (*Momordica charantia* (MC) and *Ocimmum gratissimum* (OG) were air-dried at room temperature for about two weeks. An electric blender (Kenwood blender, model KP800KA) was then used to pulverize the air-dried leaves separately and they were kept in different plastic container before the commencement of the study.

Drugs and reagents**:**

All chemicals/drugs and reagents used were of analytical grade. Drug solutions were prepared freshly before use. Donepezil and Scopolamine were manufactured by Torrent Pharma Ltd, United Kingdom and Hubei Tianyao pharmaceutical Co. Ltd. Hubei, China, respectively.

Animal and experimental design**:**

Sixty male Wistar rats weighing between 120-150 g were obtained from animal holding unit of the Department of Biochemistry, University of Ilorin. They were kept in cages in the animal house of the Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, Nigeria, and were fed with standard diet and water ad libitum. The rats were housed under standard laboratory conditions (12 hours light/dark cycle, temperature: 22 ± 3$^{o}$C) and acclimatized for two weeks before the commencement of the experiment. All the animals were strictly handled in conformation to the Declarations of Helsinki in 1995 (as revised in Edinburgh 2000) and the University's guidelines on Care and Use of Laboratory Animals.

Animal grouping and administration:

Two models were adopted for dementia induction, the scopolamine and the high fat diet models.

There were thirty (30) rats in each model. This was further divided into 6 groups, each made of 5 rats. Cognitive impairment was induced in all groups except control and were administered either donepezil, *Momordical charantia* (MC), *Ocimum gratissimum* (OG) or a combination of *Momordical charantia* (MC) and *Ocimum gratissimum* (OG).

Scopolamine model: Cognitive impairment was induced in all groups except control by a daily single injection of scopolamine (1 mg/kg, ip). One-hour post scopolamine injection, rats were administered either donepezil, *Momordical charantia* (MC), *Ocimum gratissimum* (OG) or a combination of *Momordical charantia* (MC) and *Ocimum gratissimum* (OG) as shown in Table 1. The rats were randomly divided into six groups (n = 5) as follows:

Table 1: Animal grouping

| Groups | Dosage administered to each group (daily) |
|---|---|
| A (Control) | Vehicle (5 ml/kg normal saline) orally |
| B | Scopolamine (1 mg/kg) ip. [25]. |
| C | Scopolamine (1 mg/kg) ip. + Donepezil (2 mg/kg) orally [25]. |
| D | Scopolamine (1 mg/kg) ip. + MC (400 mg/kg) orally [25]. |
| E | Scopolamine (1 mg/kg) ip. + OG (400 mg/kg) orally [25]. |
| F | Scopolamine (1 mg/kg) ip. + MC (400 mg/kg) + OG (400 mg/kg) orally [25]. |

Ip-Intraperitonally, MC- Momordical charantia; OG- Ocimum gratissimum,

High fat diet model: Cognitive impairment was induced in all groups except control by free access to a high fat diet for a period of three (3) months, after which the rats were administered either Donepezil, *Momordical charantia* (MC), *Ocimum gratissimum* (OG) or a combination of *Momordical charantia* (MC) and *Ocimum gratissimum* (OG) as shown in Table 2. These administrations were done over a period of 15 days after dementia induction. The administered drugs, MC and OG extracts were suspended in vehicle (normal saline) solution and administered orally by gastric gavage once in a day for 15 days.

Table 2: Animal grouping (High fat diet)

| Groups | Dosage administered to each group (daily) |
|---|---|
| CONTROL | Vehicle (5 ml/kg normal saline) orally |
| HFD | HFD |
| HFD Don | HFD + Donepezil (2 mg/kg bw) orally [25]. |
| HFD MC | HFD + MC (400 mg/kg bw) orally [25]. |
| HFD OG | HFD + OG (400 mg/kg bw) orally [25]. |
| HFD MC + OG | HFD + MC (400 mg/kg bw) + OG (400 mg/kg bw) orally [25]. |

HFD-High fat diet, MC- *Momordical charantia*; OG- *Ocimum gratissimum*, bw- body weight.

The drug administration was carried out between the hours of 08:00 and 10:00 in the morning. Administration of treatments regimen as shown in Table 1 and 2 lasted for fifteen consecutive days. On the last day of administration, Morris water maze (MWM) and modified dark and light box were used to assess short-term spatial memory function [26]. On day 15, rats were anaesthetized and the brain was excised and then homogenized. The supernatant was then processed for biochemical analysis of Malondialdehyde (MDA) [27], Reduced glutathione (GSH) [28], Total protein [29] and Acetylcholinesterase [30].

Preparation of the extracts**:**

A known weight of the powdered *Ocimum gratissimum* (502.6 g) and *Momordical charantia* (500 g) was macerated each in 5 litres of distilled water for 24 hours. The filtrates were dried using lyophilized freeze dryer (Freeze dryer Model: HXLG10-50DG, Hunan Kaida Scientific Instruments Co. Ltd.) which yielded 52.6g of *Ocimum gratissimum* and 48.9 g of *Momordical charantia.* The dried powders were stored in separate airtight containers till use. The calculated amount of the extract was reconstituted in normal saline to give the required doses [25].

Phytochemical screening**:**

Chemical tests were carried out on the extracts (MC and OG) using the standard procedure to identify the constituents as described by Harbone [31], Trease and Evans [32] and Sofowora [33].

Behavioral Tests:

Morris Water-Maze (MWM) Test:

Spatial memory was evaluated using the Morris' water maze [25, 26]. The maze is made up of an open circular pool of about 200 cm in diameter and 70 cm deep filled with water up to about 60 cm of the pool. A hidden platform with a top surface of about 15 cm, maintained at the same position throughout the experiment was

submerged at about 1.5 cm below the water surface. The platform was made hidden by adding milk to make the water opaque thereby creating a nearly invisible platform-to-background. First, animals were trained to locate the platform. During acquisition, trial escape latency time (ELT), time taken to locate the hidden platform, was noted as an index of learning which was recorded with the aid of a video system. Each animal was subjected to the four acquisition trials per day for 5 consecutive days before the administration.

On the last day of administration (15th day), the animals were re-exposed to the maze (to test for their spatial and long-term memory functions), a video camera was placed above the center of the pool to capture images of the swimming animal, for measures of the escape latency. The time spent by the animal in locating the hidden platform (escape latency) was noted as an index of learning.

Sample Collection**:**

On the 15th day of administration, after behavioral assessments, the rats were anaesthetized with intraperitoneal injection of ketamine (100 mg/kg). The brain tissues were isolated weighed and homogenized in 0.1 M phosphate buffer solution (pH 7.4).

The homogenate was centrifuged at 3000 rpm for 10 minutes and the supernatant were separated and used for biochemical analysis [26].

Biochemical Analysis:

Estimation of Acetylcholine esterase (AchE) level:

The cholinergic marker, acetylcholinesterase, was estimated using Acetylcholinesterase Activity Assay kit (Elabscience, China). The assay kit is an optimized version of Ellman's method [30] in which thiocholine, produced by AChE, reacts with 5, 5-dithiobis (2-nitrobenzoic acid). This homogenate was incubated for 5 min with 2.7 mL of phosphate buffer and 0.1 ml of Ellman's reagent (5, 5-dithiobis 2-nitrobenzoate, DTNB). Then, 0.1 ml of freshly prepared acetylthiocholine iodide (pH 8) was added and the absorbance was read at 412 nm.

Determination of Total brain protein**:**

Total amount of protein in brain was measured according to the method of Lowry et al. [29]. In this method under alkaline condition copper ion is reduced to form a complex, this complex then reduces folin-Ciocalteau reagent and the absorption was read at 650 nm.

Estimation of Malondialdehyde (MDA) level**:**

Malondialdehyde (MDA), marker of oxidative stress was indirectly estimated by determining the accumulation of thiobarbituric acid reactive substances (TBARS) based on the method of Mihara and Uchiyama, [27]. 0.5 ml of distilled water was added with 1 ml of 10% trichloroacetic acid and was added with 0.5 ml of brain tissue homogenate. This was centrifuged at 3000 rpm for 10 min. To the mixture, 0.1 ml of

thiobarbituric acid (0.375%) was added. Total solution was placed in water bath at 80°C for 40 min and cooled at room temperature. Absorbance was read at 532 nm.

Estimation of reduced glutathione (GSH) level:

Reduced glutathione was assayed according to the method of Ellman, [28]. The colorimetric assay involves carefully optimized enzymatic recycling method using glutathione reductase and Ellman's reagent; DTNB. Glutathione reductase reduces GSSG to GSH. DTNB (5-5-dithiobis (2-nitrobenzoic acid) reacts with GSH to form yellow colour chromophore, 5 – thionitrobenzoic acid (TNB) and GS- TNB. GS – TNB was further reduced to GSH and TNB by glutathione reductase. The absorbance was read at 415 nm and compared with standard curve for GSSG.

Statistical Analysis**:**

The results were expressed as mean ± standard error of mean (SEM). Statistical significance was done using one-way analysis of variance (ANOVA) and then subjected to post-hoc Newman-Keul test using Graph pad prism version 5. Values were considered statistically significant at $p<0.05$.

**RESULTS:**

Constituents of aqueous extracts of *Ocimum gratissimum* and *Momordica charantia* leaves is shown in table 3.

Effect of Ocimum gratissimum and Momordical charantia on the Morris water maze and biochemical parameters:

In Figure 1, the group B (scopolamine-treated) showed a significant increase in Escape Latency (EL) compared with the control group (A) (as this group of rats took a longer time to locate the hidden platform). This suggests that administration of scopolamine induced cognitive impairment in rats. Administration of aqueous leaves extracts of Momordical charantia to rats in group D, Ocimum gratissimum to rats in group E or a combination of Momordical charantia and Ocimum gratissimum, to rats in group F, each cause a significant decrease in escape latency when compared with group B (scopolamine treated; $p<0.05$). However, there was no significant difference in escape latency of the rats treated with donepezil (group C), the aqueous leaves extracts of Momordical charantia (group D).and Ocimum gratissimum (group E).

Table 3: Secondary metabolite constituents of aqueous extracts of Ocimum gratissimum and Momordica charantia leaves

| Compounds | Ocimum gratissimum | Momordical charantia |
|---|---|---|
| Saponins | Absent | Present |
| Flavonoids | Present | Present |
| Tanins | Absent | Absent |
| Phenols | Absent | Absent |
| Steroids | Present | Present |
| Terpenoids | Present | Present |
| Glycosides | Present | Present |
| Alkaloids | Present | Absent |
| Proteins | Present | Present |
| Reducing sugar | Absent | Absent |

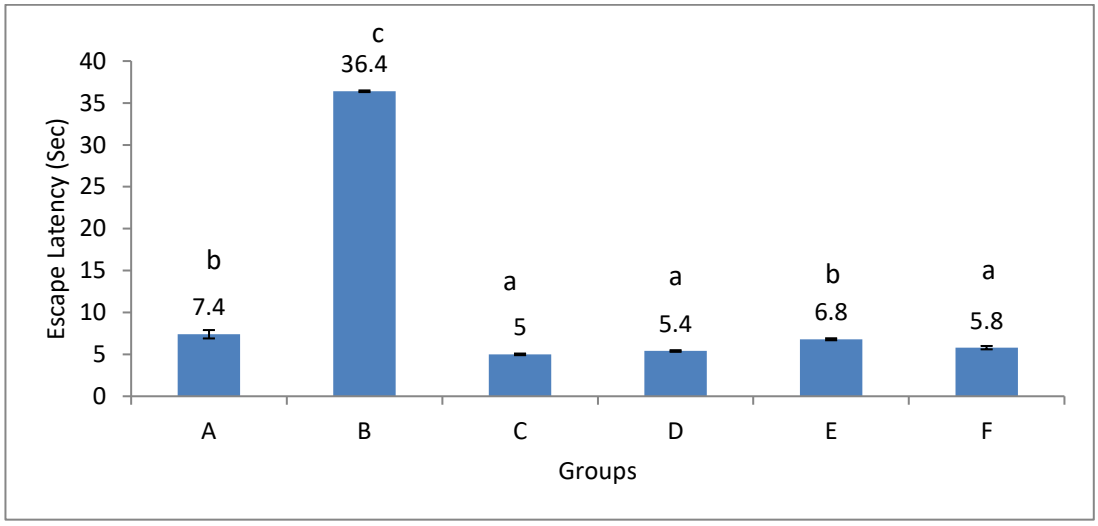


Figure 1: Effect of *Ocimum gratissimum* and *Momordical charantia* on escape latency in Morris water maze test. A - Control; B- Scopolamine treated; C-Scopolamine + Donepezil; D- Scopolamine +MC; E- Scopolamine +OG; F- Scopolamine +MC +OG. Data are presented as mean ± standard error of mean, $p< 0.05$, bar with different alphabet (superscript) are significantly different from each other

In figure 2, the total protein in the brain increased significantly in the scopolamine treated rats (group B) when compared with control (group A). Conversely, administration of aqueous leaf extract of *Momordica charantia to rats in* group D*, aqueous leaf extract of Ocimum gratissimum* to rats in group E or a combination of *Momordical charantia and Ocimum gratissimum,* to rats in group F, each cause a significant reduction in the total protein concentration in the brain ($p<0.05$) when compared with the scopolamine treated rats (group B). This is similar to the effect observed in the donepezil-treated rats (group C).

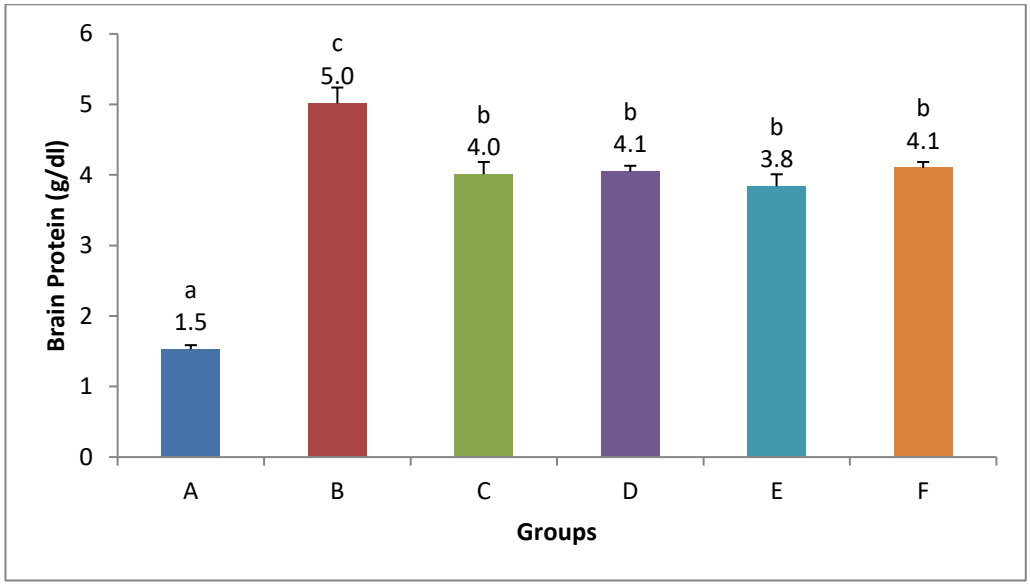


Figure 2: Effect of Ocimum gratissimum and Momordical charantia on brain protein levels. A - Control; B- Scopolamine treated; C-Scopolamine + Donepezil; D- Scopolamine +MC; E- Scopolamine +OG; F- Scopolamine +MC +OG. Data are presented as mean ±standard error of mean, $p < 0.05$, bar with different alphabet (superscript) are significantly different from each other

Figure 3 shows that the brain MDA level (an index of lipid peroxidation) was significantly increased in group B (scopolamine treated rats) when compared with the control (group A). Treatment with either aqueous leaf extract of *Momordica charantia* or/and aqueous leaf extract of *Ocimum gratissimum* (group D, E and F; $p<0.05$) significantly attenuated the level of MDA compared with the scopolamine treated rats (group B). The group E (treated with aqueous leaf extract of *Ocimum gratissimum)* compared favourably with group C (the donepezil treated rats)

The activity of AchE (an enzyme which breaks down Ach neurotransmitter) was significantly increased in the scopolamine treated rats (group B) when compared with control (group A) (figure 4). But upon administration of aqueous leaves extracts of Momordical charantia or/and Ocimum gratissimum to rats in group D, E and F, there was a significant reduction in its activity when compared with group B (the scopolamine treated rats). The reduction in the activity of AchE in the combined treatment group follow a similar pattern with group C (the donepezil treated rats). (figure 4).

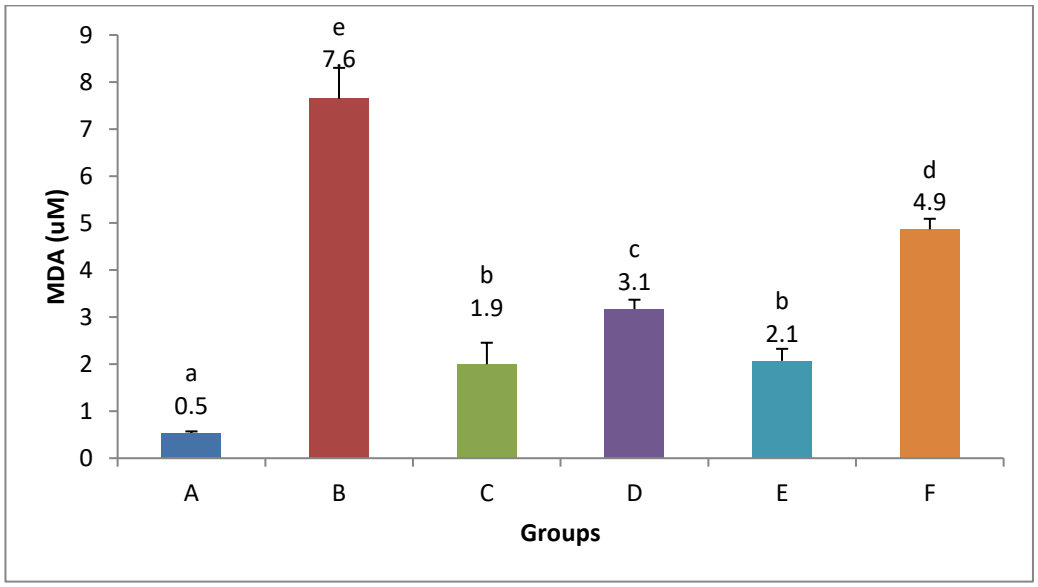


Figure 3: Effect of Ocimum gratissimum and Momordical charantia on MDA levels. A - Control; B- Scopolamine treated; C-Scopolamine + Donepezil; D- Scopolamine +MC; E- Scopolamine +OG; F- Scopolamine +MC +OG. Data are presented as mean ±standard error of mean, p< 0.05, bar with different alphabet (superscript) are significantly different from each other

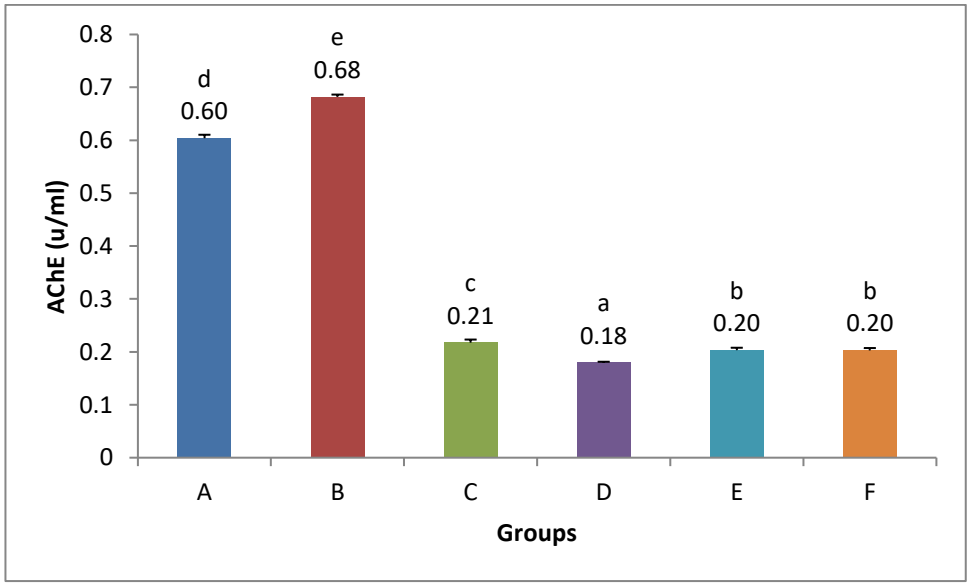


Figure 4: Effect of Ocimum gratissimum and Momordical charantia on AChE activity. A - Control; B- Scopolamine treated; C-Scopolamine + Donepezil; D- Scopolamine +MC; E- Scopolamine +OG; F- Scopolamine +MC +OG. Data are presented as mean ±standard error of mean, p< 0.05, bar with different alphabet (superscript) are significantly different from each other

In Figure 5, the rats in group B (scopolamine treated) showed an increase in the tissue level of GSH compared with group A (control rats). Administration of aqueous leaf extract of *Momordica charantia* or/and aqueous leaf extract of *Ocimum gratissimum* to rats in group D, E and F led to a more pronounced (P<0.05) increase in GSH when compared with group B (scopolamine treated). In addition, there was no significant difference in the level of GSH in the three intervention groups.

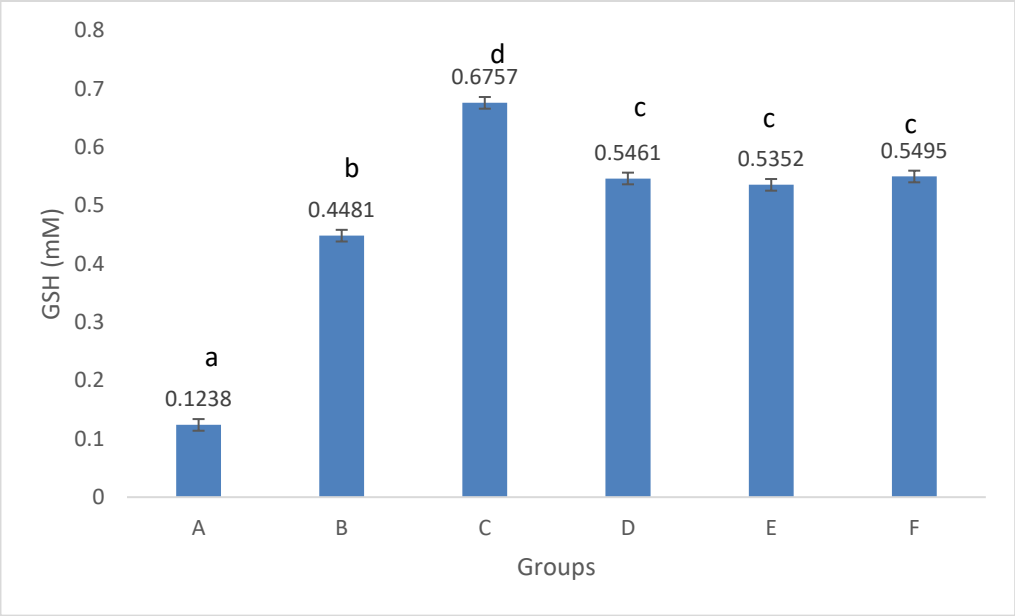


Figure 5: Effects of aqueous leaves extracts of Momordica charantia and Ocimum gratissimum on reduced glutathione (GSH) level. A - Control; B- Scopolamine treated; C-Scopolamine + Donepezil; D- Scopolamine +MC; E- Scopolamine +OG; F- Scopolamine +MC +OG. Data are presented as mean ±standard error of mean, p< 0.05, bar with different alphabet (superscript) are significantly different from each other

The rats in group B (scopolamine treated) showed a significant decrease in the level of plasma protein compared with group A (control rats) (figure 6). However, the administration of aqueous leaf extract of *Momordica charantia* or/and aqueous leaf extract of *Ocimum gratissimum* to rats in group D, E and F, led to a significant (P<0.05) increase in plasma protein level when compared with group B. The plasma protein level of the rats in group E (treated with aqueous leaf extract of *Ocimum gratissimum)* compares favorably with group C (the donepezil treated rats) figure 6.

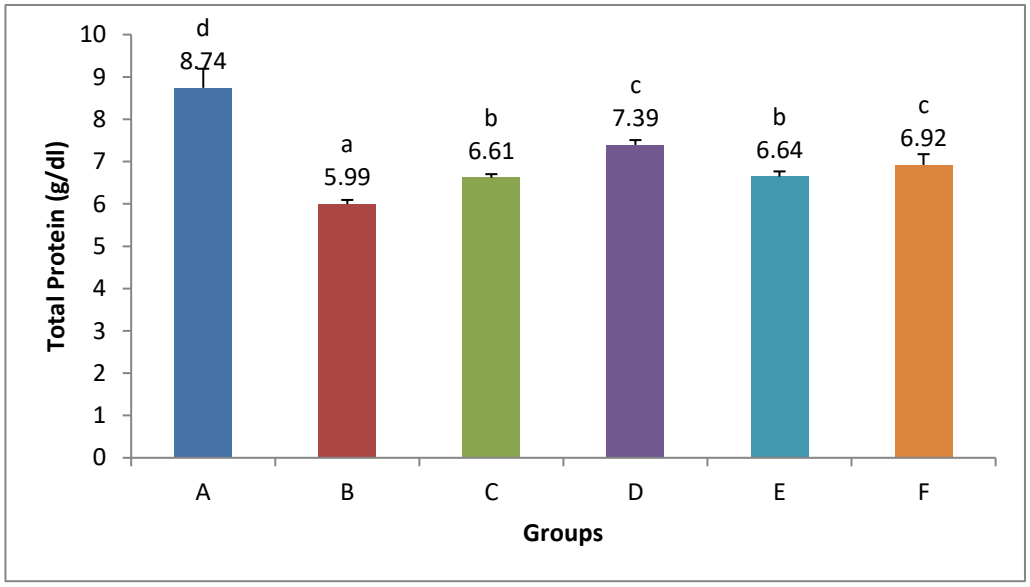


Figure 6: Effects of aqueous leaves extracts of Momordica charantia and Ocimum gratissimum on total plasma protein level. A - Control; B- Scopolamine treated; C-Scopolamine + Donepezil; D- Scopolamine +MC; E-Scopolamine +OG; F- Scopolamine +MC +OG. Data are presented as mean ±standard error of mean, p< 0.05, bar with different alphabet (superscript) are significantly different from each other

Table 4: Effects of aqueous leaves extracts of *Momordica charantia* and *Ocimum gratissimum* on Morris water maze and biochemical parameters of High fat diet-induced Spatial Memory Impairment

| Parameters/Groups | Control | HFD | HFD Don | HFD MC | HFD OG | HFD MC+OG |
|---|---|---|---|---|---|---|
| Escape latency (s) | 5.8±0.2$^{b}$ | 18.0±1.2$^{c}$ | 1.8±0.37$^{a}$ | 2.4±0.27$^{a}$ | 1.6±0.24$^{a}$ | 1.6±0.40$^{a}$ |
| GSH (mM) | 1.69±0.11$^{a}$ | 1.82±0.06$^{a}$ | 2.59±0.13$^{b}$ | 2.39±0.12$^{b}$ | 2.60±0.21$^{b}$ | 2.69±0.18$^{b}$ |
| MDA (uM) | 0.89±0.08$^{a}$ | 2.41±0.00$^{c}$ | 1.93±0.00$^{b}$ | 1.00±0.09$^{a}$ | 1.18±0.14$^{a}$ | 1.05±0.07$^{a}$ |
| Protein (mg/dl) | 2.46±0.00$^{b}$ | 2.63±0.02$^{d}$ | 2.53±0.01$^{c}$ | 2.40±0.01$^{a}$ | 2.47±0.02$^{b}$ | 2.36±0.02$^{a}$ |
| ACHE (U/ml) | 0.078±0.02$^{a}$ | 0.118±0.00$^{b}$ | 0.09±0.01$^{a}$ | 0.080±0.00$^{a}$ | 0.070±0.00$^{a}$ | 0.082±0.01$^{a}$ |
| Cholesterol | 138.2±0.58$^{b}$ | 163.4±4.7$^{c}$ | 158.8±0.97$^{c}$ | 124.2±2.24$^{a}$ | 138.8±2.47$^{b}$ | 126.2±5.52$^{a}$ |

HFD- High fat diet group, HFD Don- High fat diet+Donepezil group, HFD MC- High fat diet+*Momordical charantia* group, HFD OG- High fat diet+*Ocimum gratissimum* group, HFD MC+OG- High fat diet+*Momordical charantia*+*Ocimum gratissimum* group. Data are presented as mean ±standard error of mean, p< 0.05, mean in the same row with different alphabet (superscript) are significantly different from each other

As seen in Table 4, the HFD group exhibited a significant increase in escape latency (time taken to locate the hidden platform relative to the control. Administration of either OG or/and MC cause a significant decrease in the time taken to locate the hidden platform (decrease in Escape latency) when compared with the HFD group. Similarly, there is no significant difference in the escape latency among HFD + extract treated groups. The HFD + Donepezil Group compares favorably with all the HFD + extract treated groups.

A significant increase in the brain MDA of the HFD group was observed in this study. But upon administration of OG and MC either singly or in combination causes a significant decrease in the brain MDA when compared with the HFD group. The HFD + extract treated groups and the HFD +Donepezil groups are not significantly different $p<0.05$.

In Table 4, the HFD group showed a slight increase in the level of GSH compared with control rats (but not significant). The administration of aqueous leaf extract of *Momordica charantia* or/and aqueous leaf extract of *Ocimum gratissimum* led to a significant ($P<0.05$) increase in GSH when compared with HFD group.

Acetylcholine esterase (AchE) activity in the brain was significantly increase in the HFD group, however, administration of the aqueous extract of *Momordica charantia* or/and aqueous leaf extract of *Ocimum gratissimum* cause a decrease in acetylcholine esterase (AchE) activity similar to the HFD +Donepezil group.

The total protein in the brain increased significantly in the HFD group when compared with control group. Treatment with aqueous leaf extract of *Momordica charantia* or/and aqueous leaf extract of *Ocimum gratissimum* reduced the total protein concentration in the brain ($p<0.05$) when compared with the HFD group. The reduction in the total protein concentration in the brain. ($p<0.05$) in all the extracts (MC and OG) treatment groups followed a similar pattern with the Donepezil-treated group.

The total cholesterol level increased in the HFD group when compared with control group. Treatment with aqueous leaf extract of *Momordica charantia* or/and aqueous leaf extract of *Ocimum gratissimum* reduced the total cholesterol level ($p<0.05$) when compared with the HFD group. The reduction in the total cholesterol concentration ($p<0.05$) in all the extracts (MC and OG) treatment groups followed a similar pattern with the control group.

## DISCUSSION

Lifestyle and drug abuse are some of the risk factors of dementia. Despite enormous effort that has been directed towards the discovery of drugs that could be used in the management of dementia; there has been no definite means of treating dementia. In the present study, we assessed two established model of dementia/cognitive impairment and show that

administration of MC and/or OG prevent the spatial memory disruption induced by scopolamine or long time consumption of high fat diets in adult rats.

Specifically, our data indicated that both plants extracts were able to maintain the cholinergic activities of the brain reduce the plasma cholesterol level and prevent the oxidant-anti-oxidant imbalance induced by scopolamine or HFD. Acetylcholine is a well-studied neurotransmitter in the brain [34, 35]. It acts on the cholinergic receptors which are widely distributed in the brain to promote memory-related functions. Acetylcholine activity has been the focus of many neuroscientists in recent years [25, 26, 35]. This is because, impairment of the cholinergic transmission that result from either alteration in the levels of acetylcholine or AChE acitivity may lead to learning and memory deficit which mimics the conditions in AD patients [36,37].

Scopolamine is a nonselective acetylcholine muscarinic receptor antagonist. It induces cognitive dysfunction by disrupting the cholinergic signaling [36, 38, 39]. Scopolamine can enhance the AchE activity which in turn blocks the nerve impulses that are mediated by acetylcholine [39-41]. Studies have also shown that scopolamine administration can increase oxidative stress in the brain [40]. Increased level of oxidative stress markers has also been linked to memory deficits. MWM test was an experimental method designed by British psychologist Morris in the early 1980s [42]. Nowadays, it has become the most widely recognized method for evaluating learning and memory in rodent experiments. In the present study, we observed that treatment with scopolamine alone increases the latency to find the hidden platform in MWM experiment. This effect was reversed by the administration of donepezil. This suggests that scopolamine administration lead to impairment in spatial memory formation. Similarly, we found a significant increase in brain AChE activity in scopolamine treated rats. AChE is cholinergic enzyme which hydrolyzes ACh into acetic acid and choline. Increase AChE activity usually leads to memory dysfunction. Scopolamine can induce memory impairment by promoting brain oxidative stress. In this study we found that scopolamine-treated rats show a significant increase in brain MDA level. In this study, we observed a significant increase in the brain GSH level in scopolamine-treated group. Although, we would expect a decrease in level of GSH which is an antioxidant in nature. Instead, we observed a traumatic increase in the brain GSH level, suggesting that the enhanced oxidative stress triggers a cascade of activities to increase the brain anti-oxidant activities to prevent the brain against oxidative damage. Whether the increased brain GSH level was enough to resist the damaging effect of increased MDA/oxidative stress cannot be ascertained in this study. Collectively, memory impairment observed in the scopolamine treated rats study can be attributed to dysfunction in brain cholinergic

system and partial increase in the brain oxidative stress. Our result shows that treatment of rats with MC and/or OG prevent scopolamine-induced memory impairment, restores cholinergic dysfunction and oxidative stress. This could be due to the presence of different phytoconstituents, including flavonoids, sterols, and phenolic compounds in these plants [43,44]. Furthermore, evidence from the literature suggests that chronic HFD intake causes metabolic disorder which results in cognitive impairment, particularly in AD [45]. Different mechanisms that underlie the HFD-induced cognitive impairment in experimental animals have been reported. These include excessive production of reactive oxygen species (ROS), such as MDA and reduces the antioxidant enzyme levels [46,47].

Similarly, abnormal cholesterol accumulation has been associated with increased Aβ in cellular and most animal models of AD, and drugs that inhibit cholesterol synthesis have been shown to lower Aβ in these models [48,49]. Hence, we used HFD model which relies on comorbid effect of excessive weight gain and metabolic disorders to cause memory deficit in experimental animals and human. In our study, HFD rats shows pronounced hypercholesterolemia, increased AChE activity, increased oxidative stress as shown by enhanced MDA level in the brain, and spatial memory deficit in MWM task. Treatment with MC/OG alone or in combination did not only prevent the cognitive impairment in HFD-rats, but also reduced the plasma cholesterol level and restore the AChE activity.

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# ANDROGEN EXCESS ELICITS OXIDATIVE STRESS, INFLAMMATION AND RENAL DYSFUNCTION IN FEMALE WISTAR RATS

Running title: Androgen excess causes renal dysfunction

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**ABSTRACT**:
Hyperandrogenism has been implicated in patients with polycystic ovary syndrome, which is the most common endocrine-metabolic disorder among women of reproductive age. Hyperandrogenism has been linked with renal disorders, particularly chronic kidney disease. Therefore, the present study investigated the effect of letrozole-induced hyperandrogenism on renal function in female Wistar rats. Eight-week-old female Wistar rats were allotted into two groups (n=6 per group) which include: Control, and letrozole-treated (LET). The control group received vehicle (*p.o.*) and the LET-treated group received letrozole (1 mg/kg; *p.o.*). The administration was carried out once daily for 21 days. The results showed that the hyperandrogenic rats had increased kidney weight, metabolic profile (fasting insulin and homeostatic model of assessment of insulin resistance), renal free fatty acids, renal inflammatory biomarkers (tumor necrosis factor and interleukin-6), renal malondialdehyde, γ-glutamyl transferase, lactate production, lactate dehydrogenase, plasma creatinine and urea, and plasma and renal uric acid with a subsequent decrease in renal glutathione peroxidase. Moreover, the study revealed an increased plasma testosterone level when compared with control animals. The present study therefore indicates that letrozole-induced hyperandrogenism resulted in elevated levels of testosterone and insulin resistance which causes renal dysfunction, further accompanied by renal lipid peroxidation and inflammation.

**Keywords**: Hyperandrogenism; Kidney; Letrozole.; Polycystic ovarian syndrome
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**INTRODUCTION**:

The kidneys are important organs in the body that regulate water and electrolyte balance as well as blood pressure. Alterations of renal function, which is commonly linked to cardiometabolic and endocrine disorders, including polycystic ovarian syndrome (PCOS) have been associated with cardiovascular disorders (CVD), which remains the huge cause of death globally [1]. PCOS is an endocrine / metabolic disorder occurring in about 6–21% of reproductive-aged women [2] and is often characterized by polycystic ovaries, hyperandrogenism and anovulation / oligomenorrhea [3]. PCOS usually progresses to a number of metabolic-related pathologies, such as chronic kidney disease (CKD), which is characterized by inflammation, oxidative stress as well as endothelial dysfunction [4]. Insulin resistance (IR) is an early metabolic event in CKD [5], and this may result in end-stage renal failure with deteriorated IR. Although, the indisputable link between PCOS (hyperandrogenism)-engendered IR and CKD is unclear, several studies have linked the underlying pathogenesis to excessive lipid peroxidation and imbalance between reactive oxygen species and antioxidant enzymes [6]. Nevertheless, further investigation of its pathophysiological mechanism would provide a therapeutic target for the management of metabolic/endocrine-associated kidney disease.

The pathophysiological involvement of IR in renal disease is multifactorial in nature which is possibly secondary to perturbations that are prominent in renal diseases, including oxidative stress, chronic inflammation, metabolic acidosis and adipokine derangement. IR plays a major role in the progression of renal disease, which deteriorates renal hemodynamics by activation of the sympathetic nervous system [7]. Impaired insulin signaling has been reported as a pathogenic factor in polycystic kidney disease [8], by possibly increasing uremic toxin such as endogenous nitric oxide synthase inhibitor. This aggravates glucose dyshomeostasis in various pathological conditions [9].

Hyperandrogenism is an important criterion in diagnosing PCOS. In patients with PCOS, the rate of hyperandrogenism could be as high as 60%-80%. Androgen hyperactivation leads to ovulation disorder, menstrual disorder and acne, suggesting that hyperandrogenism is not only a clinical characteristic of PCOS, but also an important risk factor. The current anti-androgen therapies in clinical settings have not achieved satisfactory effects, which lies in the complicated mechanisms of androgen production and its wide-ranging effects [10]. The menstrual cycle abnormality is also one of the critical characteristics in patients with PCOS. Accompanied by a prolonged menstrual cycle, anovulation becomes more frequent, leading to amenorrhoea, endometrial hyperproliferation

and even carcinogenesis. Polycystic ovaries are another vital feature in women with PCOS; defined as the presentation of at least 12 antral follicles (AFC) with a diameter from 2 to 9 mm in the whole ovary and/or an ovarian volume over 10mL [11]. Excessive AFC could lead to the secretion of large amounts of oestrogen, which inhibits the secretion of follicle-stimulating hormone (FSH) via negative feedback of the gonadal axis and leads to anovulation. Therefore, the length of the menstrual cycle and the number of AFC in ovaries are the two main indicators to estimate the disease severity of PCOS. The serum level of testosterone is positively correlated with the length of the menstrual cycle and the numbers of AFC [12], suggesting that hyperandrogenism is a promoter of PCOS. In addition, excess androgen can damage granulosa cells (GCs) and change the microenvironment of follicles, resulting in follicular atresia in PCOS [13].

Chronic kidney disease (CKD) is manifested as a decrease in glomerular filtration rate (GFR; GFR < 60 mL/min) over 3 months and proteinuria. Due to the high correlation between CKD and metabolic disorders, there may be a close correlation between PCOS and kidney diseases. Previous studies revealed the presence of pre-microalbuminuria and an increase in cystatin C (a biomarker for renal function) in PCOS women [14,15]. Considering that there are abundant androgen receptors in renal cells, such as mesangial cells and proximal tubular cells, excessive androgen could be a causal risk factor for kidney diseases. Reports show that androgen/AR imposes the susceptibility to severe infections in the upper urinary tract and a high rate of urinary citrate and sodium excretion in women [16,17]. Furthermore, a significantly positive correlation between serum testosterone and renal tubular cell injury has been implicated in patients with PCOS [18], and the follicular fluid collected from patients with PCOS could induce fibrotic lesions in cultured renal proximal tubular cells [18].

However, the specific mechanism is not clear. Testosterone was also reported to induce the apoptosis in renal tubular epithelial cells, as well as necrosis via activation of hypoxia-inducible factor 1α/Bcl-2 interacting protein 3 (HIF-1α/BNIP3) pathway [19]. Another study has shown that androgen/AR and Fgf10/Fgfr2 signalling participate in renal fibrosis [20]. In addition, evidence shows that decreased androgens protect against renal injury by reducing T-cell infiltration and enhancing anti-inflammatory cytokine production [21]. Studies also show that prenatal testosterone induces proteinuria in adulthood [22], which may explain the results of pre-microalbuminuria in patients with PCOS. Although previous studies have implicated the possible molecular mechanisms of hyperandrogenism in PCOS and its related complications, necessity is laid on research to uncover its dynamic nature.

**METHODOLOGY**:

Experimental Animals and Grouping:

Eight-week-old female Wistar rats were used in this study. The rats were given unlimited access to standard rat chow and tap water. The study was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

After 2 weeks of acclimatization, the animals were randomly distributed into two groups (n=6 per group); the Control and letrozole-treated (LET) groups.

Rats were maintained in a colony under standard environmental conditions of temperature (22-26$^0$C), relative humidity (50-60%), and a 12-hour dark/light cycle. Rats were treated with letrozole (1.0 mg/kg) for 21 days as previously described [23,24,25,26].

Treatment:

Vehicle was given by oral gavage to the control group while LET group received 1.0 mg/kg of letrozole obtained from Sigma-Aldrich, in St Louis, MI by oral gavage. The treatments were done for 21 days.

Metabolic Indices:

The oral glucose tolerance test was performed 48 hours before the sacrifice of the rats:

After a 12-hour overnight fast, basal blood glucose was determined, and the rat were loaded with glucose (2.0 g/kg; oral gavage). Then blood was obtained sequentially at 30, 60, 90 and 120 minutes and the area under the curve (AUC) of glucose were monitored with a hand-held glucometer manufactured by ONETOUCH Life Scan, Inc., Milpitas, CA, USA. Insulin resistance was determined using the homeostatic model assessment of IR (HOMA-IR = fasting glucose (mmol/l)* fasting insulin (µU/l)/ 22.5) as described in the previous studies [26,27].

Collection of Samples:

Blood was collected by cardiac puncture into a heparinized tube after anesthetizing the animals with sodium pentobarbital (50 mg/kg, *ip*) and the blood was centrifuged at 704 *g* for 5 min at room temperature. Plasma was stored frozen until it was needed for biochemical assay.

Preparation of kidney homogenate:

After weighing the kidney, 100 mg section of the tissue was carefully removed and homogenized with a glass homogenizer in phosphate buffer solution, centrifuged at 8000 g for 10 min at 4 °C and the supernatant was collected and stored frozen until it was required for biochemical assays.

Biochemical analysis:

Plasma endocrine profile:

Plasma insulin and testosterone concentrations were determined with Rat ELISA kits obtained from Calbiotech Inc. in Cordell Ct., El Cajon, CA 92020, USA.

Plasma and kidney lipid profile:

Standard colorimetric methods were used to determine free fatty acids from the plasma and tissue homogenate by using assay kits obtained from Fortress Diagnostics Ltd. in Antrim, UK.

Plasma and kidney lipid peroxidation and antioxidant markers:

Malondialdehyde (MDA) is a marker of lipid peroxidation while glutathione peroxidase (GPx) assesses antioxidant capacity [28]. Malondialdehyde was determined from the plasma and tissue homogenate by standard non-enzymatic spectrophotometric method using assay kits from Randox Laboratory Ltd. in Co. Antrim, UK. Glutathione peroxidase was determined from the plasma and tissue homogenate by standard enzymatic spectrophotometric method using assay kits from Oxford Biomedical Research Inc. in Oxford, USA.

Inflammatory biomarkers:

The levels of tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) were determined in the plasma and tissue homogenate by quantitative standard sandwich ELISA technique using a monoclonal antibody specific for these parameters with rat kits obtained from Elabscience Biotechnology Inc. in Wuhan, Hubei, P.R.C., China.

Uric acid, Creatinine and Urea:

The level of plasma creatinine was determined by colorimetric method using assay kits from Randox Laboratory Ltd. in Co. Antrim, UK. Whereas plasma urea and plasma and renal uric acid concentrations were determined t by standard spectrophotometric methods, using kits from Oxford Biomedical Research Inc. in Oxford, UK.

Plasma and renal lactate, lactate dehydrogenase (LDH) and γ-glutamyl transferase (GGT):

Lactate concentration and LDH activity were determined from the plasma and tissue homogenate by standardized non-enzymatic and enzymatic colorimetric method respectively using assay kits obtained from Randox Laboratory Ltd. in Co. Antrim, UK. Whereas plasma and renal GGT activities were assayed by standardized enzymatic colorimetric method using assay kits obtained from Fortress Diagnostics Ltd. in Antrim, UK.

Immunohistochemical assessment of kidney:

Immunohistochemical evaluation of renal tissue was performed using an inflammasome antibody (NLRP3) obtained from Elabscience Biotechnology Inc. in Wuhan, Hubei, P.R.C., China, in adherence to the procedures described in the previous study [28].

Data analysis and statistics:

All data were expressed as means ± S.D. Statistical group analysis was performed with

Graphpad Prism software version 9. Student T-test was used to compare the mean values of variables between the groups. Statistically significant differences were accepted at $p<0.05$.

Ethical approval:

The study was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and was approved by the Institutional Ethical Review Committee of Afe Babalola university.

Consent to participate is not applicable.

**RESULTS**:

Effect of LET-induced hyperandrogenism on kidney weight in animal model: There was a significant increase in the kidney weight of letrozole-induced hyperandrogenic animals compared to the control animals (Figure 1).

Effect of LET-induced hyperandrogenism on metabolic profile in animal model: Metabolic profiles such as fasting insulin and HOMA-IR significantly increased in experimental hyperandrogenic animals compared with the control (Figure 2).

Effect of LET-induced hyperandrogenism on plasma and renal free fatty acids in animal model: There was a significant increase in the renal but not plasma FFA in experimental hyperandrogenic animals compared with the control animals (Figure 3).

Figure 1: Effect of letrozole induced hyperandrogenism on kidney weight in LET-induced PCOS. Data are expressed as means ± S.D., n=6. (*$p<0.05$ vs. CTL; #$p<0.05$ vs LET). Control (CTL); Letrozole (LET).

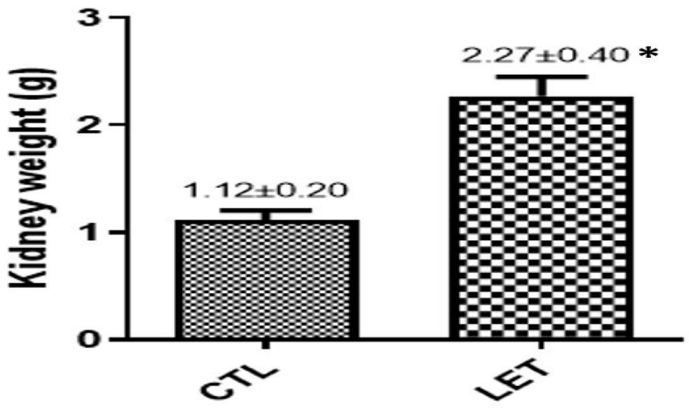


Figure 2: Effect of letrozole induced hyperandrogenism on metabolic profile: fasting blood glucose (a) fasting insulin (b) and HOMA-IR (c) in LET-induced PCOS. Data are expressed as means ± S.D., n=6. (*p<0.05 vs. CTL; #p<0.05 vs LET). Control (CTL); Letrozole (LET); Homeostatic model of assessment of insulin resistance (HOMA-IR).

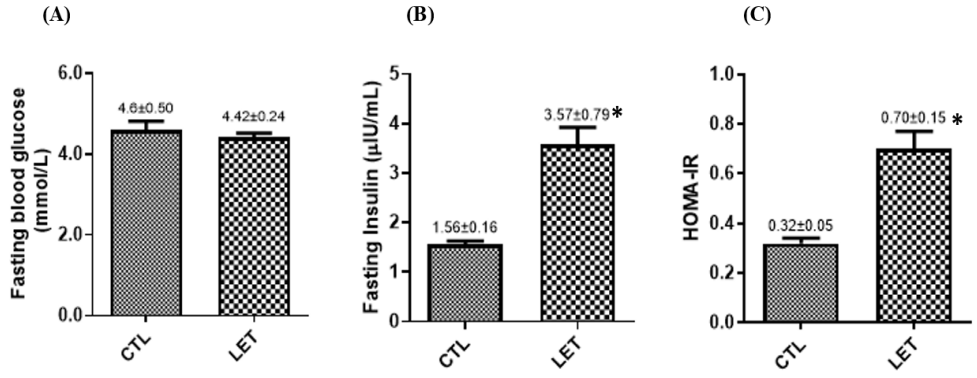
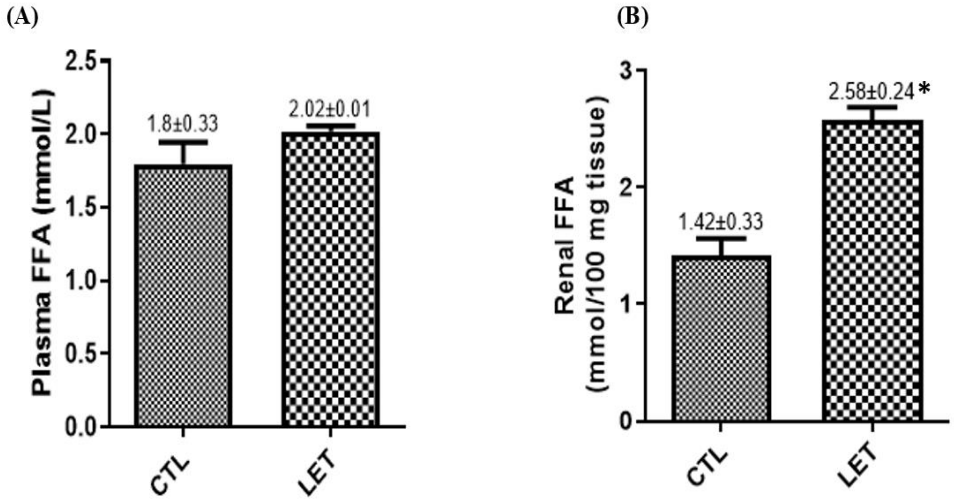



Figure 3: Effect of letrozole induced hyperandrogenism on lipid profile: plasma FFA (a) and renal FFA (b) in LET-induced PCOS. Data are expressed as means ± S.D., n=6. (*$p<0.05$ vs. CTL; #$p<0.05$ vs LET). Control (CTL); Letrozole (LET); Free fatty acid (FFA).




Effect of LET-induced hyperandrogenism on renal inflammatory biomarkers in animal model: Inflammatory biomarkers such as TNF-$\alpha$ and IL-6 increased significantly in the renal tissue of experimental hyperandrogenic animals compared with the control (Figure 4).

Effect of LET-induced hyperandrogenism on renal malondialdehyde, g-glutamyl transferase

and glutathione peroxidase in animal model: In experimental hyperandrogenic animals, there was a significant increase in renal MDA and GGT while GPx decreased significantly in the kidney compared to control animals (Figure 5).

Effect of LET-induced hyperandrogenism on renal lactate and lactate dehydrogenase in animal model: There was a significant increase in lactate and LDH in experimental hyperandrogenic animals when compared with the control (Figure 6).

Effect of LET-induced hyperandrogenism on plasma and renal electrolytes in animal model: There was a significant increase in the plasma and renal uric acid, plasma creatinine and urea in experimental hyperandrogenic animals compared with the control animals (Figure 7).

Effect of LET-induced hyperandrogenism on plasma testosterone in animal model: There was a significant increase in the plasma testosterone levels in experimental hyperandrogenic animals compared with the control animals (Figure 8).

Figure 4: Effect of letrozole induced hyperandrogenism on renal TNF-α (a) and renal IL-6 (b) in LET-induced PCOS. Data are expressed as means ± S.D., n=6. (*$p<0.05$ vs. CTL; #$p<0.05$ vs LET). Control (CTL); Letrozole (LET); Tumor necrotic factor-α (TNF-α) Interleukin-6 (IL-6).

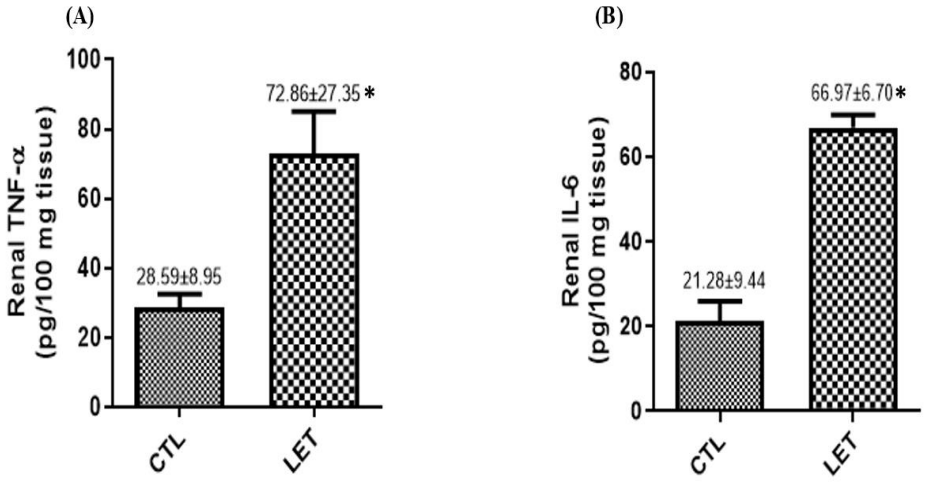


Figure 5: Effect of letrozole induced hyperandrogenism on lipid peroxidation: renal MDA (a) renal GPx (b) and renal GGT (c) in LET-induced PCOS. Data are expressed as means ± S.D., n=6. (**p<0.05* vs. CTL; *#p<0.05* vs LET). Control (CTL); Letrozole (LET); Malondialdehyde (MDA); Glutathione peroxidase (GPx); Gamma Glutamyl transferase (GGT).

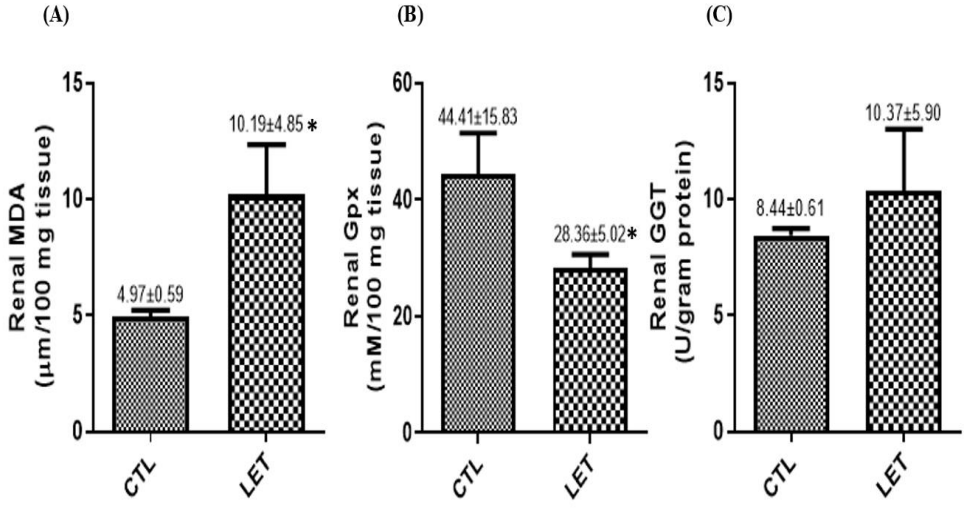



Figure 6: Effect of letrozole induced hyperandrogenism on lipid peroxidation: renal lactate (a) and renal LDH (b) in LET-induced PCOS. Data are expressed as means ± S.D., n=6. (**p<0.05* vs. CTL; *#p<0.05* vs LET). Control (CTL); Letrozole (LET); Lactate Dehydrogenase (LDH).

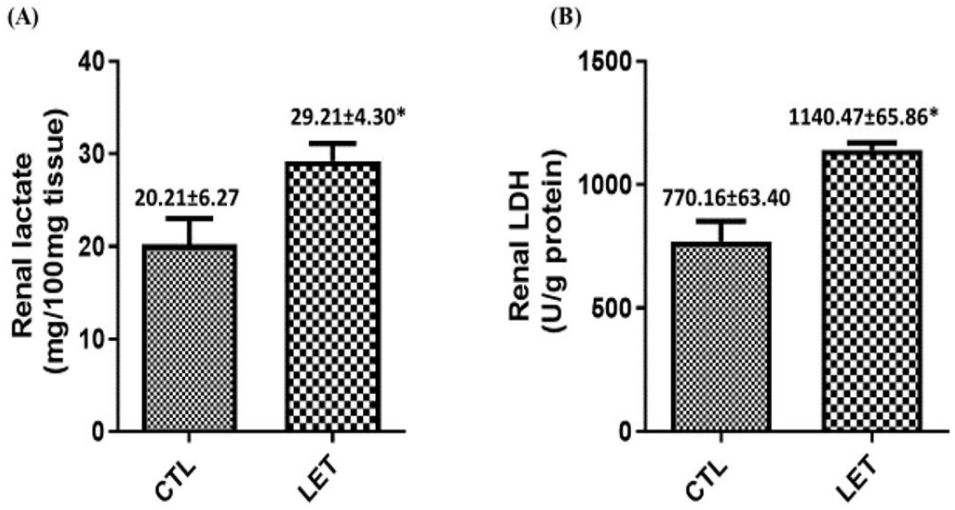


Figure 7: Effect of letrozole induced hyperandrogenism on renal electrolyte: plasma uric acid (a) renal uric acid (b) plasma creatinine (c) and plasma urea (d) in LET-induced PCOS. Data are expressed as means ± S.D., n=6. (*$p<0.05$ vs. CTL; #$p<0.05$ vs LET). Control (CTL); Letrozole (LET).

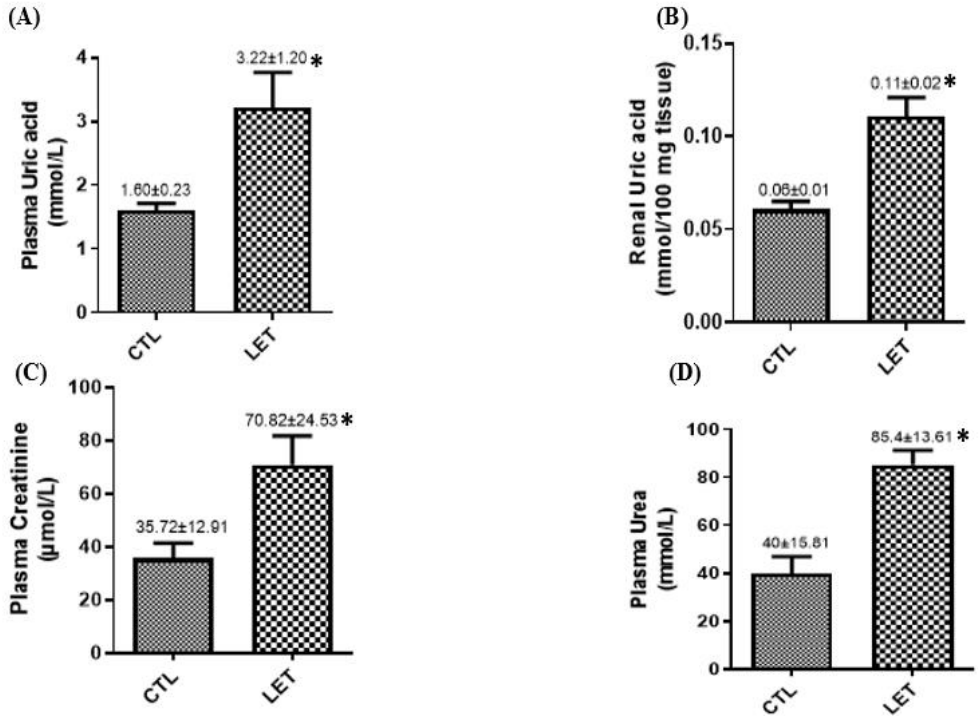



Figure 8: Effect of letrozole induced hyperandrogenism on plasma testosterone level in LET-induced PCOS. Data are expressed as means ± S.D., n=6. (*$p<0.05$ vs. CTL; #$p<0.05$ vs LET). Control (CTL); Letrozole (LET).

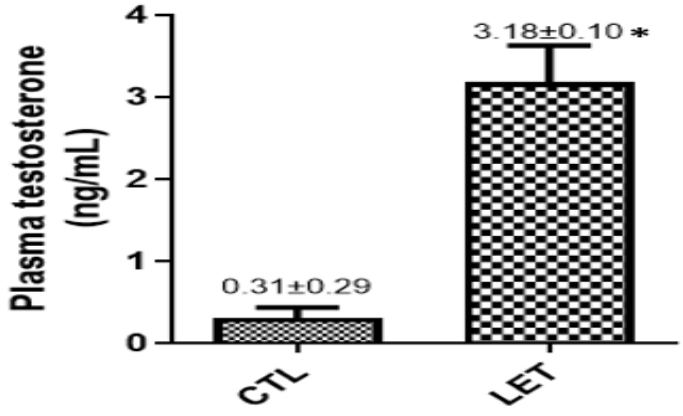
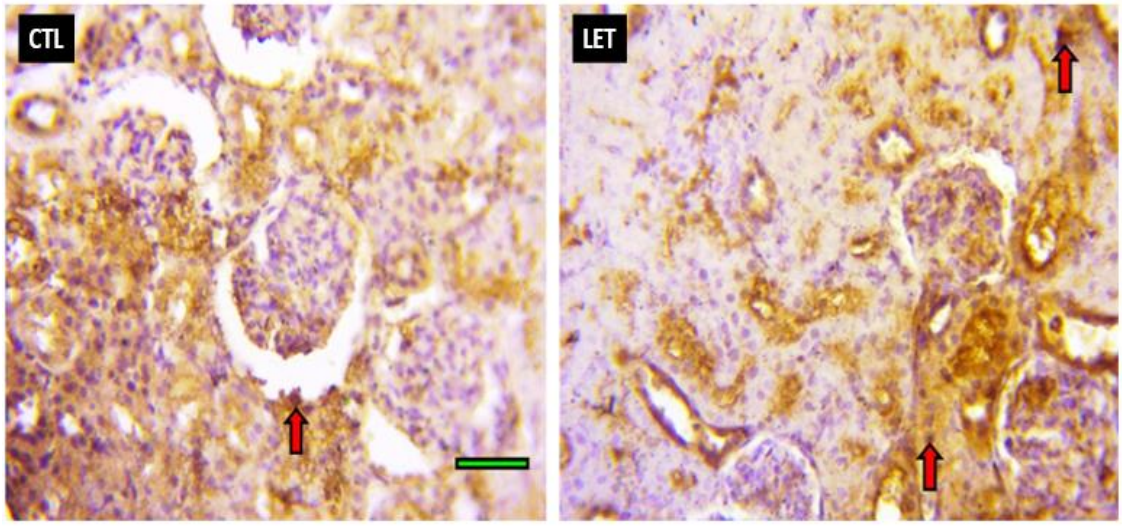


Figure 9: Immunohistochemical staining for inflammasome (NLRP3) antibody in the renal section. Control groups showed no expression of inflammasome (a) and letrozole-induced hyperandrogenic animal showed severe expression of inflammasome (b). Scale bar: 51 µm (red arrow indicate a positive response to inflammasome antibody).




Immunohistochemical staining for inflammasome (NLRP3) antibody in the renal section:

Immunohistochemical staining for inflammasome (NLRP3) antibody in the renal section. Control groups showed no expression of inflammasome. However, letrozole-induced hyperandrogenic animal showed severe expression of inflammasome (Figure 9).

**DISCUSSION**:

The novel finding in this present study is that hyperactivity of androgen hormones which is a well-known characteristic of PCOS can affect renal functions. PCOS has a close association with the progression of metabolic abnormalities such as obesity, diabetes and hypertension, which are the major causes of kidney disease [29,30]. These suggest PCOS may have an intimate association with kidney injury. Few studies have investigated the underlying mechanism of PCOS-associated kidney injury. In this study, we have established a link in PCOS with kidney injury through experimental research.

To test the hypothesis that PCOS is associated with kidney injury, we evaluated kidney function in two group of female Wistar rats, PCOS induced with letrozole and control. The present study provides different metabolic risks, and IR is a hallmark of the classic hyperactivation of androgens through increased free fatty acid. Obesity is strongly associated with PCOS [31]. Although, obesity is not a diagnostic criterion for

PCOS, both obese and non-obese PCOS patients have more visceral adipose tissue (VAT) than that of women without PCOS, and VAT has been positively correlated with total androgen levels suggesting obesity plays a crucial role in PCOS [32]. Compared with normal-weight PCOS patients, overweight PCOS patients exhibit significantly higher serum-free testosterone and free androgen indices [33]. All evidence indicates that androgens are closely related to obesity in PCOS patients. However, current data on the association between hyperandrogenism and obesity are limited and controversial. Abdominal obesity is a condition of relative hyperandrogenism. Androgens have been shown to induce abdominal adipose accumulation [34] and may cause adipose tissue dysfunction, including increased lipid accumulation and insulin resistance [35,36]. Lipogenic enzymes and antilipolytic genes are overexpressed in the omental adipose tissue of women with PCOS compared with that in non-hyperandrogenic women, meaning that androgens may play an important role in adipose lipid accumulation [36]. Androgens have also been implicated in insulin-mediated glucose dysmetabolism in adipose tissue. Previous study observed insulin resistance in subcutaneous adipose tissue in PCOS women, due to elevated levels of Testosterone (T), which inhibits insulin-mediated glucose uptake via impairment of phosphorylation of protein kinase C zeta in obese women [35]. Obesity, particularly abdominal obesity, along with its accomplice insulin resistance, also aggravates hyperandrogenism [37]. Obesity mainly manifests as increased levels of free fatty acids (FFAs), cholesterol, triglycerides and various apolipoprotein abnormalities [38]. Increased FFAs decrease insulin sensitivity and reduce glucose uptake in intramyocellular lipids [39]. FFAs can also activate serine/threonine kinases and ultimately decrease the tyrosine phosphorylation of IRS-1. Those reactions can promote insulin resistance [40]. Abdominal obesity and insulin resistance cooperatively stimulate excess androgen synthesis in the ovaries as well as the adrenal glands [41], with subsequent increases in abdominal obesity and inflammation, thus creating a pathological cycle. In addition, adipocytes further produce leptin and adiponectin via paracrine and autocrine glands, in order to regulate androgen levels in circulation. Serum leptin is increased in some PCOS patients, and high leptin concentrations inhibit the expression of aromatase mRNA in GCs [39], thus preventing the conversion of androgens to oestrogens, leading to increased serum androgens levels and ultimately promoting follicular atresia. Adiponectin is secreted by adipose tissue and is one of the most important adipose factors. Adiponectin has been observed to improve insulin sensitivity, which in turn reduces FFA intake as well as gluconeogenesis. Shorakae and team reported high adiponectin levels, which were negatively correlated with the free-androgen index and

fasting insulin. These were observed to be lower in women with PCOS than in non-PCOS women [42] Thus, concluding the diverse impact of obesity on androgen levels via various pathways.

Most studies have focused on the lipid nephrotoxicity hypothesis based on Moorhead's work [43]. Scholars who buttress this hypothesis believe that inflammation could be attributed to hyperlipidemia. However, to date, the cellular and molecular mechanism associating hyperandrogenism with renal disease is limited. Lipid accumulation triggers renal dysfunction through excessive ROS production. One of the main sites for renal lipid accumulation is the renal proximal tubule cells. High levels of albumin-bound long-chain saturated fatty acids are known to promote the progression of renal tubular damage and interstitial fibrosis; excess of Ox-HDL induced pro-inflammatory pathways, including TNF-α and IL-6, and increased the production of ROS [44,45]. PCOS causing increased FFA leads to chronic inflammation and develop severe renal degeneration and glomerular damage, indicating that the aggravation of obesity may itself exacerbate existing kidney damage, perhaps due to the overexpression of TNFα, IL-6, and monocyte chemotactic protein-1 (MCP-1) during inflammation, which results in the thickening of the glomerular basement membrane, extracellular matrix, glomerulosclerosis [46]. In particular, lipid accumulation can initiate ER stress to enhance TNFα or IL-6 in HMC and HK2 cells, resulting in the increased production of ROS and direct toxic effects on the kidney [46]. Elevated levels of ROS markers will aggravate endothelial dysfunction and vascular disease in CKD and cause an increase in uremic toxins uric acid and creatinine [47]. As a result of oxidative stress levels being increased, it is naturally bound for lipid peroxidation level (MDA and GSH) to increase and lipid antioxidant level will decrease [48]. As a result of insulin resistance causing hyperglycaemia, lipid peroxidation increases. However, due to increase in insulin resistance, naturally lactate and lactate dehydrogenase are bound to be elevated which was pointed out in the result [49].

**CONCLUSION**:

The present study therefore indicates that letrozole-induced hyperandrogenism resulting in elevated levels of testosterone and insulin resistance causes renal dysfunction, which is accompanied by renal lipid peroxidation and inflammation.

Conflict of interests: The authors declare no conflict of interest

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**SHORT COMMUNICATION:**

# ZINC SUPPLEMENTATION FOR TREATMENT OF SARS-COV-2 INFECTIONS

Short Running Title: Zinc for SARS-CoV-2 infections

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**ABSTRACT**:
The COVID-19 pandemic disproportionately affects older people, who have a higher risk of severe infections and mortality. Zinc is a readily available supplement that is gaining interest as potential treatment for SARS-CoV-2 infections. Zinc deficiency is associated with susceptibility to infections. People with COVID-19 infections have a reduced level of serum zinc compared to controls. Zinc supplementation has been shown to be helpful for people with respiratory tract infections through multiple mechanisms including improving viral clearance. A study on community zinc supplementation found a lower rate of symptomatic COVID-19 infections compared to control. The use of zinc in critically unwell COVID-19 patients was associated with reduced 30-day mortality. A study of COVID-19 infected inpatients showed that zinc reduced the rate of needing intensive care, while outpatient treatment may reduce symptom duration by three days. Given the higher rate of adverse outcomes in older people, zinc supplementation should be further evaluated and considered for treatment of SARS-CoV-2 infections.

**Keywords**: COVID-19, immunity, older people, zinc

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**Zinc supplementation for treatment of SARS-CoV-2 infections**:

As of late 2019, the world faced an unprecedented coronavirus disease 2019 (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. Older people are a vulnerable group and are disproportionately affected by COVID-19 infections. Approximately half of the older patients affected by COVID-19 infections have severe infections, one in five become critically unwell and a one in ten die [2]. Trials are ongoing to evaluate the efficacy of specific treatment, ranging from antimalarials, such as chloroquine, and antivirals such as lopinavir/ritonavir and remdesivir to reduce the rate of these adverse outcomes [3].

Zinc supplementation is gaining interest as potential therapy for individuals with SARS-CoV-

2 infections due the pre-existing evidence of its role in the development and function of the immune system against viral infections [3-5]. Zinc is inexpensive, readily available globally, can be administered orally and has minimal side effects [5]. Individuals with suboptimal zinc levels were found to have an associated higher risk of infections, autoimmune disorders and cancer [5]. Prolonged zinc deficiency may contribute to an imbalance in the immune system, with a higher susceptibility to infections with severe zinc deficiency [3].

A cohort study identified a significant reduction in circulating serum zinc in COVID-19 patients compared to healthy controls [6]. Serum zinc levels were also associated with disease severity, with severe COVID-19 infections having lower serum zinc levels [6]. In Asian populations, there were no significant correlations observed between zinc deficiency and COVID-19 deaths; however, there was a significant positive correlation between zinc deficiency prevalence and COVID-19 cases per million population in Asian countries [7]. While the exact role of zinc in SARS-CoV-2 transmissibility is unclear, it is hypothesised to be related to its antiviral properties of inhibiting RNA synthesis, topoisomerase and viral replication [4].

Administering zinc supplements in individuals with respiratory tract infection has been shown to reduce symptom severity, frequency and duration of the infection [5]. Zinc supplementation has also been proposed by research and clinical groups to manage patients with SARS-CoV-2 infections [8]. This is because zinc may play a role in improving mucocilliary clearance of virus particles, supporting integrity of the respiratory epithelia, reducing viral replication, conserving antiviral immunity, reducing hyperinflammation risk, facilitating anti-oxidative effects and thus minimising lung damage and secondary infections [5].

A prospective single-blinded study conducted in 2020 during the peak of the COVID-19 pandemic evaluated the effects of oral zinc supplementation in a community with circulating SARS-CoV-2 [8]. Participants were treated with daily doses of 10 mg, 25 mg, or 50 mg zinc picolinate. Although there were no differences in the COVID-19 symptomology of participants between the three treatment groups, individuals administered zinc picolinate were less likely to develop symptomatic COVID-19 infections compared to the control group [8]. These findings suggest that zinc supplementation even at 10mg daily doses may be beneficial in alleviating severity of COVID-19 infections in the community.

A 2021 retrospective study observed an association between the use of zinc sulphate as adjunctive therapy with significantly lower 30-day mortality in critically ill patients with COVID-19 infections [9]. Survival benefits were also

observed in these patients after they were prescribed zinc supplements within 30 days of their hospital stay [9]. A 2022 prospective randomised double-blind placebo-controlled multicentre trial reviewed the efficacy of zinc supplementation in COVID-19 inpatients and outpatients [10]. In addition to standard supportive care, patients were given 25 mg elemental zinc twice daily for 15 days or placebo and were evaluated over a 30-day period [10]. There was a significant decrease in the risk of Intensive Care Unit (ICU) admission within 30 days, and a shorter duration of COVID-19 symptoms in the inpatient group treated with zinc supplementation compared to placebo. For the outpatients, zinc supplementation reduced the duration of COVID-19 symptoms by approximately 3 days. These studies support zinc supplementation to treat patients with COVID-19 infections who seek medical treatment in outpatient and inpatient settings, including critically unwell patients.

The optimal formulation and administration route of zinc for COVID-19 infections requires further evaluation. For instance, the cellular uptake of zinc can be improved when combined with a zinc ionophore such as hydroxychloroquine [11]. In a 2020 retrospective observational study of COVID-19 inpatients not requiring ICU care, treatment with zinc sulphate combined with hydroxychloroquine and azithromycin was associated with reduced mortality or transition towards palliative care [11]. However, whether the addition of zinc ionophore is superior than that of zinc alone has yet to be determined.

In summary, there are several studies demonstrating the benefits of zinc in the management of SARS-CoV-2 infections in multiple settings, ranging from community supplementation during a pandemic, outpatients, inpatients and critically ill patients. This may be a useful therapeutic approach to consider for older people, who are at a higher risk of severe infections and mortality. Further research is required to elucidate the role of zinc in SARS-COV-2 infections and the optimal dose, formulation and preferred route of administration.

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