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Highlights

Reduced Ejection Fraction (HFrEF)

Rural African Patient with Heart Failure

Discovering Thoughts, Inventing Future

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The use of LCZ-696(Sacubitril/Valsartan) and SGLT2inhibitors: A Real World Experience in a Rural African Patient with Heart Failure with Reduced Ejection Fraction (HFrEF). A Case Series

By Dominick Mkombozi Raphael, Gerald Jamberi Makuka, Abdu Hussein Mogella, Beatrice Kabuka, Rosemary Thadeus Mushi & Collins Boamah

St. Francis University of Health and Allied Science

Abstract- *Objective:* To observe the outcomes of the use of both Angiotensin Receptor-Neprilysin inhibitor (ARNI) and sodium-glucose cotransport 2-inhibitor (SGLT2Ii) in terms of echocardiographic parameters, clinical symptoms, cardiovascular death, and Heart failure hospitalization in patient with heart failure reduced ejection fraction (HFrEF) in the hard-to-reach rural area of Africa.

Background: Angiotensin Receptor-Neprilysin inhibitor (ARNI) is preferred over angiotensin-converting enzymes inhibitor or an angiotensin II receptor blocker as foundation therapy for patients with heart failure with reduced ejection fraction to reduce the risk of cardiovascular death, Heart failure hospitalization, and Heart failure symptoms. SGLT2 inhibitor (Dapagliflozin and Empagliflozin) is among the four foundation drugs in managing HFrEF.

Keywords: *HFrEF; sacubitril/valsartan; SGLT2i; Rural Africa Population.*

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THEUSEOFLCZ696SACUBITRILVALSARTANANDSGLT2INHIBITORSAREALWORLDEXPERIENCEINARURALAFRICANPATIENTWITHHEARTFAILUREWITHREDUCEDEJECTIONFRACTIONHFREFACASESERIES

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The use of LCZ-696(Sacubitril/Valsartan) and SGLT2inhibitors: A Real World Experience in a Rural African Patient with Heart Failure with Reduced Ejection Fraction (HFrEF). A Case Series

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Abstract- Objective: To observe the outcomes of the use of both Angiotensin Receptor-Neprilysin inhibitor (ARNI) and sodium-glucose cotransport 2-inhibitor (SGLT2i) in terms of echocardiographic parameters, clinical symptoms, cardiovascular death, and Heart failure hospitalization in patient with heart failure reduced ejection fraction (HFrEF) in the hard-to-reach rural area of Africa.

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Method: A case series of 3 patients with HFrEF. Here we report a case series of three patients with HFrEF associated with Hypertension (cases #1, 2), and one postpartum dilated cardiomyopathy (case #3). The first case was a 49-year-old male, and the second was a 61-year-old male, third was a 34-year-old female. After previous ineffective treatment, the administration of ARNI and SGLTI led to a rapid and marked improvement of the clinical conditions in all three cases. All cases underwent a baseline ECHO, and 6–month follow-up time, very few patients were reported in this case report series due to economic reasons. Most of the patients cannot afford the costs of these drugs.

Results: In case number 1(case#1) the was an increase of (Left ventricular ejection fraction (LVEF) by 33.3% at –months. There was a reduction of (Left ventricular end-diastolic diameter (LVEDD) by 20% at six months, and the clinical symptoms were improved. All patients had function class I NYHA at a time of follow-up. There was one episode of Heart failure hospitalization, but there was no CV death. In case number 2(case #2), we observed a significant increase in LVEF by 75%, and a reduction of LVEDD by 14.3% at the 6-month follow-up. In the case number 3 (case#3) a patient with postpartum dilated cardiomyopathy, we observed a significant increase in LVEF from 15% to 45% at 6-month, and a reduction of LVEDD by 31.25%. The patient had a significant improvement in diastolic dysfunction from grade III to grade II. All patients showed improvement in LV wall dysfunction, and decreasing in valve regurgitation severity from severe regurgitation to mild regurgitation.

Conclusion: These data demonstrate the efficacy and safety of combining ARNI and SGLT2 inhibitors as among the four foundation drugs in HFrEF in improving morphofunctional remodeling parameters, clinical symptoms, preventing cardiovascular death, and Heart failure hospitalization in rural African patients with HFrEF.

Keywords: HFrEF; sacubitril/valsartan; SGLT2i; Rural Africa Population.

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I. Introduction

Heart failure is a highly prevalent condition, over 600 million people have heart failure; this, is more than 5x the number of cancer patients globally, which means a 1 in 5-lifetime risk of developing Heart failure for people at 40 years old [1]. Heart failure is associated with a high rate of morbidity and mortality; 50%, of HFrEF patients will die within five years of diagnosis [2]. The most recent publication in rural Africa in Tanzania, involving 812 participants, revealed a high prevalence of Hypertension 66%, Left ventricular hypertrophy 42%, severe systolic Heart failure 22%, Hypertensive Heart disease (41%), Valvular heart disease (18%), Coronary heart disease (18%), Peripartum cardiomyopathy (7%), other non-hypertensive dilated cardiomyopathies (6%) in adults, and congenital heart disease (34%) in children [3]. Symptoms and signs of heart failure are attributed to the inability of the heart to produce sufficient cardiac output

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[4]. The classification of Heart failure based on Left ventricular ejection fraction resulted in four subtypes, and among of subtypes is Heart failure with reduced left ventricular ejection fraction (HFrEF) with ejection fraction (EF) of ≤40%; [5-6].

A large, Phase III randomized clinical trial, PARADIGM-HF (Prospective Comparison of ARNI With ACEI to Determine Impact on Global Mortality and Morbidity in Heart Failure), showed massive improvements in the outcome with Sacubitril/Valsartan, changes in clinical symptoms (NYHA), LVEF, reduction of NT-proBNP, Heart failure hospitalization and CV death [7]. The DAPA-HF, EMPEROR Reduced, and DELIVER study both trials revealed benefits in clinical outcomes with Sodium-glucose Cotransporter-2 (SGLT2) Inhibitors and ARNI in patients with HFrEF when added as one of the foundation drugs in the HFrEF. Within one month in the DAPA-HF trial, a reduction in the primary outcome of a composite of worsening Heart failure or cardiovascular death with Dapagliflozin was rapidly apparent, with a sustained significant benefit at 28 days [8]. SGLT2 inhibitor either Empagliflozin or Dapagliflozin is recommended to be used in patients with HFrEF, with or without accompanying Type 2 Diabetes (T2DM), to improve HF symptoms and quality of life and to reduce the risk of HF hospitalization and, or CV death [9]. Despite advancements in medical equipment, procedures, and treatments, Heart failure management is complex and remains a challenge to Healthcare providers. The cost of the drugs for four foundation therapies (ARNI+MRA+SGL2i+BB) of heart failure management, especially ARNI and SGL2i, makes it difficult for rural Africans to afford these medications.

a) *Pathophysiology of Heart failure and consequences of reduced Ejection F*

Reducing in left ventricular ejection fraction activates a cascade of adaptive mechanisms to maintain adequate cardiac output [10]. The RAAS and adrenergic system are activated; the activated RAAS results in increases sodium and water retention, while activated adrenergic system which leads to increased left ventricular contractility and vasoconstriction [10-11]. Neuroendocrine is started to meet cardiac output demand, but continuous activation results in poor adaptation and cardiac remodeling; these, affects Left ventricular function ability to meet metabolizing tissue demands [12]. The free circulating levels of angiotensin-II have been shown to increase in heart failure patients, which impacts cell function, impair intrinsic myocardial contractility, increase ventricular stiffness, and impair diastolic function [13]. Sympathetic activation causes eccentric hypertrophy of the left ventricular, remodeling, and worsening heart failure [14].

b) *Mechanism of LCZ-696(Sacubitril/valsartan)*

Sacubitril/valsartan (LCZ-696) is a combined Neprilysin inhibitor and angiotensin AT1 receptor blocker [15]. Sacubitril is a prodrug, and the therapeutic effect of Sacubitril/Valsartan is partly achieved via the action of the active metabolite of Sacubitril, which inhibits Neprilysin. At the same time, blockade of the Angiotensin II type 1 (AT1) receptor is provided by the action of valsartan [16-17].

c) *Mechanism of SGLT2 Inhibitors on cardiovascular effect*

The mechanism of SGLT2 inhibitors in Heart failure works by constriction of renal afferent arteriolar that results in a reduction of intraglomerular pressure hence causing diuresis, glycosuria, natriuresis, and proteinuria; thus results in a decrease of preload, reduction in Left ventricular wall stress, decrease in afterload, reduction of HbA1c level (due to glycosuria), reduce blood pressure, and increase cardiac output (Ejection fraction) [18-20]. SGLT2 inhibitors decrease visceral fat area, subcutaneous fat area, total fat area, lower triglyceride level and LDL-C without interfering with HDL/LDL ratio, significantly increase plasma adiponectin levels, and improve endothelial microvascular dysfunction [21-23].

Despite awareness concerning Sacubitril/ valsartan and SGLT inhibitors and their efficacy when used for the managing of HFrEF, there are no published studies on efficacy, safety, and outcomes of Sacubitril/Valsartan with SGLT2I in the real-world African rural population. Therefore, this case series aim to show how the rural African population, which was underrepresented in most trials globally, responds to the novel drug ARNI and SGLTI together on the clinical symptoms, echocardiographic parameters, and cardiovascular outcomes (CV death and HF hospitalization) in HFrEF patients.

We report 3 cases with HFrEF who could afford these drugs and were successfully treated with four foundation drugs of HFrEF (ARNI+MRA+SGL2i+BB) regime in our daily practice and breaking off a long series of hospitalization episodes and prevented CV morbidities and mortality.

Case Reports #1

Patient history

Patient #1 was a 49-year-old male known case of Hypertension and type 2 diabetes on irregular medication, none smoker, and none alcohol drinker. He presented to the emergency department because of progressive dyspnea, palpitation, fatigability, and lower limb edema. He was admitted and started on 3L of oxygen via nasal cannula, spironolactone 12.5mg orally daily and intravenous (IV) furosemide to relieve congestion. On examination he was obese grade 2, with high Blood Pressure 170/92mmHg, Pulse Rate 110bpm

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(60-102), lower limb edema grade 3, elevated jugular venous pressure (JVP 7cm), Apex beat on the 5-intercostal space lateral to the midclavicular line, S3-Gallops sound, bilateral basal coarse crepitation, and anterior crackles. His Laboratory workup reveals elevated FBG 150.3mg/dl (65-95mg/dl), Hb1AC 7.7 % (<5.7%), LDL-C 160mg/dl (62-130mg/dl), Total cholesterol 230mg/dl(0-200mg/dl), HDL-C 21mg/dl(<40-0mg/dl), Hemoglobin(Hb) 15.4g/dl(13.3-16.2), white blood cell (WBC) 5.0 X1000/mcL (3.54-9.06), serum Creatinine 1.3mg/dl(0.5-1.5mg/dl), BUN 15mg/dl(7-20), Potassium 4.0mmol/L(3.5-5.0), Sodium 139mmol/L, ALT(SGPT) 15U/L(7-41), AST(SGOT) 24U/L(12-38). (NT-proBNP, hsTnT, and Troponin T were not tested). The electrocardiogram (ECG) examination showed features of Left ventricular hypertrophy with sinus tachycardia Heart rate 110bpm. Echocardiography studies revealed severe concentric left ventricular hypertrophy with severe heart failure, Ejection fraction 36%, Left ventricular end-diastolic dimension (LVEDD) was 54mm, E/A 2.2, E/e/ 15), LAVI 46/mm2, DT(ms) 106(>200ms), TAPSE 14mm secondary mitral regurgitation moderate–severe, normal leaflets, mild tricuspid regurgitation, Global hypokinesia, inferior vena cave was dilated, and none-collapsing and estimated pulmonary arterial pressure was 33 mmHg, and there was no sign of thrombus. Chest x-ray revealed pulmonary edema, bilateral mild pleural effusion, and Left ventricular enlargement.

The patient was diagnosed with Heart Failure, reduced Ejection Fraction [HFrEF] NYHA Class III with Diastolic failure grade III, T2DM, and dyslipidemia. He was kept on IV Furosemide 80mg 6hrly, and then he had a weight loss of more than 1.5kg, and urine output was 5L/day. Furosemide was switched to orally route 40mg three times daily, Spironolactone 12.5mg, Lisinopril was added 2.5mg once daily then adjusted on the third day to 5mg once daily, and before discharge Carvedilol 3.125mg twice daily was added, Clopidogrel 75mg once daily, Atorvastatin 40mg (high-intensity management) once daily, Metformin 1g twice daily. He was discharged on the 5th day after being admitted. He was instructed to come for a clinic visit and doses titration after two weeks and potassium and creatinine monitoring after two weeks.

Seven days post-discharge, he started experiencing paroxysmal nocturnal dyspnea, orthopnea, nocturnal cough, and an increase in lower limb edema, and he had functional class III NYHA. The patient was hospitalized again, on examination he was dyspneic grade 3, desaturation on room air with SPO_2 90%, Blood Pressure 156/97mmHg, Pulse rate 109bpm (60-100 bpm) regular-regular, pitting edema grade 3, gallops sound on the cardiac exam and bilateral fine and coarse crackles anterior chest and posterior. The plan of management was changed as follows: kept on oxygen with nasal cannula 4L/min, Lisinopril was stopped for three days before starting ARNI, and Metformin was stopped to Dapagliflozin 10mg once daily, carvedilol was adjusted to 6.25mg twice daily, spironolactone was adjusted to 25mg once daily, IV Furosemide was given stating dose of 120mg then reduced to 80mg IV three times daily plus Metolazone 5mg once daily. On the fourth-day post admission, ARNI was initiated at a dose of 25mg twice daily. So the patient was kept on the evidence-based treatment guidelines with ARNI+MRA+SGLT2i+BB+DIURETIC and was discharged on the 7th day.

Two weeks later, the patient came back for a follow-up; he, was stable, had no severe Heart failure symptoms, only minimal fatigability on exercise, functional class I NYHA, serum potassium (K+) was 3.7mmol/L (3.5-5.0), and serum creatinine was 1.2mg/dl (0.5-1.5mg/dl. The doses were titrated as follows: Adjusted ARNI 50mg twice daily, Spironolactone 25mg once daily, dapagliflozin 10mg once daily, Atorvastatin 40mg (high intensity), Furosemide 40 mg three times daily, Metolazone 5mg once daily, and carvedilol 12.5mg twice daily.

After one month (4 weeks follow-up), he came for a follow-up, he reported tremendous improvement, and he had no symptoms and signs of heart failure to disclose. Blood Pressure was 134/87mmHg, Pulse Rate 69bpm (Target <70-60), serum potassium (K+) 3.7mmol/L (3.5-5.0), FBG 75.3mg/dl (65-95mg/dl). He was planned to continue with a similar therapeutic plan, but still Metolazone was stopped, Furosemide was changed to 40mg twice daily, and ARNI was adjusted to 100mg twice daily, but other medications remained the same. The patients continued throughout all the clinic visits without up-titrating the doses of medicine because of hypotension and economic challenge. So he was maintained on the following therapeutic plan: ARNI 100mg twice daily, Spironolactone 25mg once daily, Dapagliflozin 10mg once daily, Carvedilol 12.5mg twice daily, Furosemide 40mg twice daily, Atorvastatin 40mg once daily, Clopidogrel 75mg once orally daily.

At six months (24 weeks follow up), the baseline investigations were performed. Blood Pressure 125/78mmHg, Pulse rate 69bpm, FBG 68.1mg/dl (65-95mg/dl), Hb1AC 4.9 % (<5.7%) LDL-C 65mg/dl (62-130mg/dl), and Total cholesterol 163.2mg/dl(0-200mg/dl), HDL-C 32mg/dl(<40-0mg/dl), Hemoglobin(Hb) 14.4g/dl(13.3-16.2), white blood cell (WBC) 5.3 X1000/mcL (3.54-9.06), serum Creatinine 0.9mg/dl(0.5-1.5mg/dl), BUN 12mg/dl(7-20), Potassium 4.4mmol/L(3.5-5.0), Sodium 136mmol/L, ALT(SGPT) 21U/L(7-41), AST(SGOT) 26U/L(12-38)), at echocardiography, Left ventricular end-diastolic dimension (LVEDD) was 45mm, indicating hypertrophy, with minimal wall thickness and left ventricular ejection fraction (LVEF) was increased to 48%. No mitral insufficiency was found, all valves were standard, and the left atrium was slightly enlarged. The inferior vena cava (IVC) was normal. The estimated pulmonary arterial

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pressure was 30 mmHg. So he was maintained on the following therapeutic plan: ARNI 100mg twice daily, Spironolactone 25mg once, Dapagliflozin 10mg once daily, Carvedilol 12.5mg twice daily, Torsemide 10mg once daily, Atorvastatin 40mg once daily, and Clopidogrel 75mg once daily.

Case Reports #2

Patient history

Patient #2 was a 61-years-old male known case of Hypertension, CKD stage3B, T2DM, old cerebral infarction, and lateral myocardial infarction, and not on regular medication for three months, a smoker and none alcohol drinker. He presented as the emergency department because of progressive dyspnea, palpitation, nocturnal cough, fatigability, and lower limb edema but no chest pain and no night sweat. He was admitted and started on 4L/min of oxygen via nasal cannula. Still, his SPO_2 rose from 78% to 86%, and then was changed to a simple face mask 10L/min, which rose SPO2 from 86% to 97% as well as Eplerenone 25mg orally daily and intravenous (IV) furosemide 120mg to relieve congestion. On examination, he was confused, restless, obese grade 3, with high Blood Pressure 230/127mmHg, Pulse rate 117bpm (60-102), ascites, lower limb pitting edema grade3, elevated jugular venous pressure (JVP 10cm pulsating and easily occluded by finger), apex beat on the 6-intercostal space lateral to the midclavicular line, S1 and S2 heard with regurgitation murmur at the mitral and tricuspid, bilateral basal coarse crepitation and reduced air entry on lower lobes bilaterally. His Laboratory workup reveals elevated FBG 205.8mg/dl (65-95mg/dl), Hb1AC 9.5 %(<5.7%) LDL-C 196mg/dl (62-130mg/dl), and Total cholesterol 253mg/dl(0-200mg/dl), HDL-C 19mg/dl(<40-0mg/dl), Hemoglobin(Hb) 12.4g/dl(13.3-16.2), Mean Corpuscular Volume (MCV) 81.8fL(79-93.3), Ferritin 200(0-300) Platelet count 339 x1000/mcL(165-415), white blood cell (WBC) 4.3.0 X1000/mcL (3.54-9.06), serum Creatinine 2.3mg/dl(0.5-1.5mg/dl), BUN 35mg/dl(7-20), Potassium 4.6mmol/L(3.5-5.0), Sodium 136mmol/L(136-146), ALT(SGPT) 19U/L(7-41), AST(SGOT) 31U/L(12-38), Albumin 4.2g/dl(4.0-5.0), C-RP 15(0-5), hSTnT, TnT and NT-proBNP were not done. The electrocardiogram (ECG) examination showed features of Left ventricular hypertrophy with sinus tachycardia (Heart rate 122bpm), ST Elevation on Lead 1, aVL, and V5-V6. Echocardiography studies revealed concentric left ventricular hypertrophy with severe heart failure, LVEF 20%, left ventricular end-diastolic dimension (LVEDD) was 54mm, IVSd (10.4mm), PWd (8.7mm), LVIDs (53mm), LVOT (21.36mm), RA (71ml), LA (41mm), LA (68mL), LAVI (43.00ml/m2), TAPSE 10.00, Sa (7.6cm/s), E/A 0.33), E/e/ 5.88, DT(ms) (130.00), secondary mild mitral regurgitation (EROA 11.00mm2), Vmax (423cm/s), VTI (95.60cm), normal anterior leaflet, lateral wall dysfunction, inferior vena cave was dilated and none-collapsing, and not estimated pulmonary pressure. Chest x-ray revealed pulmonary edema, bilateral mild pleural effusion, and enlarged both right ventricle and left ventricle.

The patient was diagnosed with Heart Failure, reduced Ejection Fraction [HFrEF] NYHA Class III, CKD stage 3B, T2DM and Dyslipidemia, old cerebral infarct (ischemic stroke), and lateral myocardial infarction. He was kept on IV Furosemide 80mg 6hrly, and then he had a weight loss of more than 2.5kg, and urine output was 4.5L. Furosemide was reduced to 40mg IV TDS, Eplerenone 25mg, Enalapril was added to 2.5mg twice daily then adjusted on the third day to 5mg twice daily for two weeks, and before discharge Bisoprolol, 5mg once daily was added, DAPT (Aspirin 75mg and Clopidogrel 75mg once orally daily), Atorvastatin 80mg (high-intensity management), Emplagliflozin 10mg once daily. He was discharged on the 8th day after being admitted. He was instructed to come for clinic follow-up and dose titration after two weeks, plus monitoring of serum potassium and creatinine.

Two weeks later, he came back for clinic follow up; he reported to have minimal improvements, although for the past seven days since discharged he had been experiencing progressive dyspnea at night, nocturnal cough, lower limb edema, and fatigability despite using all the medications as instructed by the discharging doctor. On assessment, he had functional class III NYHA. The patient was hospitalized again, on examination he dyspneic grade 2, desaturation on room air with SPO_2 93breath/min, Blood Pressure 187/107mmHg, Pulse rate 102bpm (target <70-60) regular-regular, pitting edema grade 2, regurgitation murmur at mitral and tricuspid valves on the cardiac exam and on Respiratory system he had bilateral fine crackles. The plan of management was changed as follows: kept on oxygen with nasal cannula 4L/min, Enalapril was adjusted to 10mg twice daily, Bisoprolol was adjusted to 10mg once daily, Eplerenone remained the same 25mg once daily, IV Furosemide was given stating dose of 120mg then 80mg three times daily plus Metolazone 10mg once daily. On the fourth-day after discharge, he reported that he had been experiencing easy fatigability, dry cough, and shortness of breath. On examination, the Blood Pressure was 170/97mmHg. He was hospitalized and Enalapril was stopped for one week before initiating ARNI. One week later, ARNI was initiated at a dose of 25mg twice daily and continued with DAPT (Aspirin 75mg and Clopidogrel 75mg once orally daily both).There is no Catheter lab in our setting, and referral to the tertiary hospital was not possible due to unstable clinical condition and financial problem)). So the patient was kept on the evidence-based treatment guidelines with ARNI+MRA+SGLT2i+BB+DIURETIC and continued with other drugs for other comorbidities and was discharged on the 12thday post admission.

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Two weeks later, the patient came back for a follow-up. He was stable, had no severe Heart failure symptoms, functional class II NYHA, lower limb edema grade 2, Blood Pressure 167/102mmHg, Pulse rate 98bpm(target <70-60), serum potassium (K+) was 4.8mmol/L (3.5-5.0), and serum creatinine was 1.1mg/dl (0.5-1.5mg/dl. The doses were up-titrated, ARNI 50mg twice daily, Eplerenone 25mg once daily, Dapagliflozin 10mg once daily, Atorvastatin 80mg (high intensity) once daily, Furosemide 40 mg twice daily, stopped Metolazone 5mg, Bisoprolol 10mg once daily. The ESC 2021 HFrEF management guideline recommends the addition of Isosorbide dinitrate +Hydralazine to Africans with heart failure whose blood pressure is not controlled, so Isosorbide Dinitrate 20mg three times daily and Hydralazine 37.5mg three times daily was added. After one month (4 weeks follow-up) follow-up, he reported tremendous improvement. Blood Pressure was 131/80mmHg, Pulse rate 70bpm (target <70-60), serum potassium (K+) 4.7mmol/L (3.5-5.0), FBG 85.5mg/dl (65-95mg/dl). So he was maintained on the following therapeutic plan: ARNI 100mg twice daily, Eplerenone 25mg once daily, Dapagliflozin 10mg once daily, Bisoprolol10mg once daily, Furosemide 40mg twice daily, Atorvastatin 80mg once daily, Clopidogrel 75mg once daily, Aspirin 75mg once daily, Isosorbide Dinitrate+Hydralazine 20/37.5mg three times daily.

At six months (24weeks follow up) the baseline was performed; Blood Pressure 135/98mmHg, Pulse rate 69bpm, FBG 78.1mg/dl (65-95mg/dl), Hb1AC 5.2 % (<5.7%), LDL-C 85mg/dl (62-130mg/dl), and Total cholesterol 190mg/dl(0-200mg/dl), HDL-C 37mg/dl(<40-0mg/dl), Hemoglobin(Hb) 13.4g/dl(13.3-16.2), Mean Corpuscular Volume (MCV) 86.4fL(79-93.3), Ferritin 150(0-300) white blood cell (WBC) 4.8 X1000/mcL (3.54-9.06), serum Creatinine 1.4mg/dl(0.5-1.5mg/dl), BUN 10mg/dl(7-20), Potassium 4.9mmol/L(3.5-5.0), Sodium 140mmol/L(136-146), ALT(SGPT) 21U/L(7-41), AST(SGOT) 22U/L(12-38)), at echocardiography, left ventricular end-diastolic dimension (LVEDD) was 46mm, lateral wall akinesia, and left ventricular ejection fraction (LVEF) was increased to 35%, TAPSE 18, mild mitral insufficiency was found, mild tricuspid insufficiency, and the left atrium was mild enlarged, LAVI 38.0ml/m^2. The inferior vena cava (IVC) was normal. No Estimated pulmonary arterial pressure. So the doses were adjusted as follows: ARNI 100mg twice daily, Eplerenone 50mg once daily, Dapagliflozin 10mg once daily, Bisoprolol 10mg once daily, Furosemide 40mg twice daily, Isosorbide Dinitrate +Hydralazine 20/37.5mg three times daily, Atorvastatin 80mg once daily, Aspirin 75mg once daily, and Clopidogrel 75mg once daily. The target LDL-C recommended in a very high-risk ASCVD patient is <1.4mmol/L[<55mg/dl], so this patient did not achieve his target LDL-C which is why Ezetimibe 10mg once daily was added on high intensity statin. He was advised to have salt reduction due to his recurrent dyspnea, and also to have minimal physical activities and continue with monthly clinical follow-up.

Case Reports #3

Patient history

Patient #3 was a 34-year-old female unknown case of Hypertension and none alcohol drinker; she, presented to the emergency department because of asthenia, dyspnea with moderate exertion, fatigability, lower limb edema, paroxysmal nocturnal dyspnea, nocturnal cough. She was three (3 months) post spontaneous vertex delivery of a twin pregnancy. On examination, she was dyspnea grade 3, SPO_2 93% on room air .Still, she was kept on oxygen therapy 2L/min with a nasal cannula, with high Blood Pressure 127/92mmHg, Pulse rate 130bpm (60-102) irregular-irregular, lower limb edema grade 3, elevated jugular venous pressure (JVP 9cm), Apex Beat on the 6-intercostal space lateral to the midclavicular line, mitral regurgitation murmur, bilateral basal coarse crepitation. Her Laboratory workup reveals, Hemoglobin(Hb) 15.4g/dl(13.3-16.2), white blood cell (WBC) 5.0 X1000/mcL (3.54-9.06), serum Creatinine 1.2mg/dl(0.5-1.5mg/dl), BUN 8mg/dl(7-20), Potassium 4.5mmol/L(3.5-5.0), Sodium 135mmol/L(136-146), ALT(SGPT) 19U/L(7-41), AST(SGOT) 28U/L(12-38). NT-proBNP was not done. The electrocardiogram (ECG) examination showed features of Left ventricular hypertrophy and atrial fibrillation (HR 132bpm). Chest x-ray revealed pulmonary edema, bilateral mild pleural effusion, and globular heart. Echocardiography studies revealed severe eccentric left ventricular hypertrophy with severe Heart failure, LVEF 15%, ventricular end-diastolic dimension (LVEDD) was 63mm, IVSd (7.00mm), PWd (10.00mm), LVIDs (64mm), LVOT (20.00mm), RA (61ml), LA (42mm), LA (64mL), LAVI (43.00ml/m2), D-sign on the regional wall, Mild pericardial effusion, TAPSE 15.00, Sa (11.00cm/s), FAC (31), MPI (1.33), E/A 2.16, E/e/ 21, DT(ms) 72, mild secondary mitral regurgitation, normal anterior and posterior leaflet, Vmax(430cm/s), VTI (94.00cm), mild tricuspid regurgitation, inferior vena cave was dilated, and none-collapsing and an estimated pulmonary arterial pressure were (39.00mmHg), and there was no sign of thrombus.

The patient was diagnosed with Postpartum dilated cardiomyopathy with severe Heart Failure reduced Ejection Fraction [HFrEF] NYHA Class III, Diastolic dysfunction Grade III, and Paroxysmal atrial fibrillation (Afib). She was kept on IV Furosemide until she had a weight loss of 1.5kg and urine output was 5L. IV Furosemide was switched to orally route 40mg three times daily. The patient was discharged after one week of hospitalization, with the following therapeutic plan: Spironolactone 12.5mg once daily, ARNI 25mg twice daily, Dapagliflozin 10mg once daily, Rivaroxaban 15mg

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once daily, Amiodarone 800mg twice daily for two weeks then 400mg twice daily for two weeks, Metoprolol 50mg once daily. She was instructed to come after two weeks for the follow-up clinic for dose titration and to monitor serum potassium and serum creatinine. Bromocriptine was not initiated because she could not afford an alternative feeding for her twin babies.

Two weeks later, the patient came back for a follow-up. She was stable, Heart failure symptoms functional class II NYHA, Blood Pressure 110/70mmHg, Pulse rate 100bpm irregular-irregular, serum potassium (K+) was 3.9mmol/L (3.5-5.0), and serum creatinine was 1.4mg/dl (0.5-1.5mg/dl, eGFR 50.6ml/min/1.73m2, CrCl 47ml/min, and resting ECG showed similar findings. The doses were up-titrated as follows: Adjusted ARNI 50mg twice daily, Spironolactone 25mg once daily, Furosemide 40mg twice daily, Dapagliflozin 10mg once daily, Rivaroxaban 15mg once daily (not adjusted because the CrCl was <50ml/min), Amiodarone 400mg twice daily, Metoprolol 100mg once daily.

After one month (4 weeks follow-up), she came for a follow-up. She reported much improvement, she had Heart failure symptoms class I NYHA, BP was 108/77mmHg, Pulse rate 96bpm (Target <70-60) irregular-irregular, serum potassium (K+) 4.7mmol/L (3.5-5.0), serum creatinine was 1.2mg/dl (0.5-1.5mg/dl, eGFR 60.9ml/min/1.73m2, CrCl 55ml/min. A dynamic ECG Holter examination showed an average heart rate of 102 bpm, atrial fibrillation, no ventricular couplet, and no isolated ventricular ectopic (VE). The doses were up-titrated as follows: Adjusted ARNI 100mg twice daily, Spironolactone 25mg once daily, dapagliflozin 10mg once daily, Rivaroxaban 15mg once, Amiodarone 400mg twice daily (was continued for long-term rhythm control), Metoprolol 100mg once daily, Ivadradine was added 2.5mg twice daily (because the HR was still >70bpm) on the maximum dose of Metoprolol, and Furosemide was reduced to 40mg once daily.

At six months (24 weeks follow up), the baselines were performed; Blood Pressure 105/78mmHg, Pulse rate 80bpm regular-regular rhythm, Hemoglobin(Hb) 14.0g/dl(13.3-16.2), white blood cell (WBC) 5.9 X1000/mcL (3.54-9.06), serum Creatinine 1.2mg/dl(0.5-1.5mg/dl), BUN 11.2mg/dl(7-20), Potassium 4.6mmol/L(3.5-5.0), Sodium 140mmol/L(136-146), ALT(SGPT) 29U/L(7-41), AST(SGOT) 18U/L(12-38), Resting ECG examination showed features of Left ventricular hypertrophy and presences of P-wave, regular-regular rhythm, no feature of atrial fibrillation. The Echocardiography studies showed normal inferior vena cava, revealed moderate eccentric left ventricular hypertrophy with mildly reduced ejection, EF 45%, ventricular end-diastolic dimension (LVEDD) was 44.00mm, IVSd (10.00mm), PWd (6.00mm), LVIDs (37mm), LVOT (20.00mm), RA (23ml), LA (37mm), LA (35mL), LAVI (19.00ml/m2), hypo-kinesia on the regional wall motion, no pericardial effusion, TAPSE (25.00mm), Sa (18.00cm/s), FAC (33), MPI (1.23), E/A (1.50), E/e/ 26, DT(ms) 185.00(>200ms), mild secondary mitral regurgitation, EROA (7.00mm2), normal anterior leaflet, VTI (202.00cm), Vmax (616.00cm/s), no tricuspid regurgitation, and pulmonary arterial pressure was not estimated(no tricuspid regurgitation). So the doses were adjusted as follows: ARNI 100mg twice daily, Spironolactone 50mg once daily, Dapagliflozin 10mg once daily, Metoprolol 100mg once daily, Furosemide 40mg once daily, Ivabradine 5mg twice daily, Rivaroxaban 15mg once daily, and stopped Amiodarone. She was advised to have sodium reduction (advised on table spoon salt strategy), to avoid pregnant until she has completely recovered, good drugs adherence, and regular cardiac clinic follow-up.

II. Discussion

Heart failure occurs after decrease in cardiac output which triggers over-activation of different compensatory mechanisms in the body that include: increased sympathetic nerves activation, increased activation of RAAS, and increased vasoconstriction due to conversion of angiotensin I to angiotensin II. The continuous activation of these compensatory mechanisms leads to hypertrophy and ventricular remodeling that result in a decrease of cardiac output [24]. All our patients showed better outcomes when they were initiated on four foundation therapies of HFrEF (ARNI+MRA+BB+SGLT2I) that we blocked Sympathetic nervous system activation (Norepinephrine) with Beta-blockers, we blocked Renin-Angiotensin-Aldosterone System (RAAS) with ARNI, and blocked the Angiotensin pathway with mineral ocorticoid receptor antagonists[25,26]. The current recommended evidence-based SGLT2 inhibitors with good cardiovascular outcomes in Heart failure patient with or without type 2 diabetes (T2DM) are either Dapagliflozin which is mainly used in HFrEF and HFpEF, Empagliflozin, mainly used in Heart failure regardless of Ejection Fraction spectrum, and Sotagliflozin, which is used primarily in worsening Heart Failure[27,28]. The addition of either of SGLT2 inhibitors to our patients resulted in a tremendous improvement in clinical symptoms especially on the edema, blood pressure, sugar(HbA1c), left ventricular wall stress, decreasing afterload, decreasing preload and increasing LV ejection fraction (EF%)[29].

In a patient with acute heart failure features the use of diuretics is recommend to start with, in our cases all patients presented with acute decompensation heart failure with the symptoms of congestion that included lower limb edema, pulmonary edema, elevated jugular venous pressure, hepatomegaly, and ascites, that's why diuretics were initiated first [30]. The initiation of diuretics to our patients helped to improve their clinical

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symptoms, the use of loop diuretics (furosemide) and mineralocorticoid receptor antagonists, and SGLT2 inhibitors showed a significant impact in reducing congestion and decreasing preload [31,32].

Additional treatment strategies are needed to further decrease the risk for patients with acute Heart failure and for those with worsening Heart failure from getting poor Cardiovascular outcomes [32]. The European Society of Cardiology recommended the addition of Metolazone to loop diuretic if decongestion in patient with acute Heart failure is not achieved [33]. In case number 2 of this report we managed to achieve decongestion and good diuresis after adding Metolazone as a thiazide diuretic to loop diuretic without any mortality [34, 35].

The addition of Isosorbide Dinitrate and Hydralazine to ARNI in case number 1(case#1) helped to reduce the resistance Hypertension and improved Cardiovascular outcomes as it was shown to other studies. The use of ISDN/Hydralazine is indicated in African American or African patients with Heart failure who have uncontrolled Arterial Blood pressure on maximum tolerable of ACEI/ARB/ARNI or who are intolerance to ANRI/ACEI/ARB [36-38].

Dilated cardiomyopathy is associated with a high prevalence of different types of atrial fibrillation, as seen in our patient case number 3(case#3), who presented with paroxysmal atrial fibrillation [26]. The presence of Atrial fibrillation (AFib) in HFrEF makes the Heart failure outcomes even worse [39]. We controlled the rhythm of our patient with Amiodarone which is only indicated in Afib patients with structural heart disease [40]. For our case, the rhythm at the time of follow-up (at six months) was restored into a normal sinus rhythm which contributed to improved cardiovascular outcomes in this patient [41, 42]. None-vitamin K Oral Anti-Coagulant (Rivaroxaban) was used to prevent stroke in our case; although, this patient needed a long-term follow-up but at the six-month follow-up, there was no incidence of stroke or transient ischemic attack reported, the use of anti-coagulant in AFib is indicated to prevent cardio-embolic stroke if the patient has mitral regurgitation and aortic stenosis or regurgitation [43]

The Cardiovascular (CV) outcomes in our cases were improved when ARNI and SGLT2 inhibitors were initiated, but after initiation of ARNI, there was no HF hospitalization [44]. CV Death was not reported during follow-up time.All patients achieved good CV outcomes, and the combination of ARNI and SGLT2I did not harm the patients but reduced morbidity and mortality [45, 46].The patients in this case reported were either attended at St. Francis Referral Hospital (SFRH) or Good Samaritan Cancer Hospital (GSCH). Sacubitril/Valsartan is available at GSCH pharmacy only in our local setting. One tablet of ANRI costs USD 4.3 and needs to be taken twice daily, making it even harder for ordinary rural dwellers to afford this medicine, and the cost of Dapagliflozin/Empagliflozin is USD 1.5. Most rural African patients diagnosed with HFrEF qualify to use ARNI and SGLT2inhibitor, but few of them afford, and sometimes the patient can afford the drug for one month only.

III. Conclusion

This case report broke the gap in the uncensored data and shows that using of both ARNI and SGLT2 inhibitors in the rural African population with heart failure-reduced ejection fraction (HFrEF) was efficacious and safe. This suggests that using of both agents together could further lower morbidity and mortality in patients with HFrEF in the rural African population. Using of ARNI and SGLT2I in preventing cardiovascular death and Heart failure hospitalization events in rural African population with HFrEF patients with different characteristics and comorbidities was similar to other races who were involved in different real-world trials and studies. A large observational study or randomized trial is recommended for rural African populations with long-term follow-up on using of both ARNI and SGLT2I in HFrEF patients.

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Declaration of interest

The authors declare no conflict of interest to declare.

Ethical statement

Since it was a case report, ethical clearance was waived, and the data were all fully anonymized before being accessed. The study followed the principles of the Declaration of Helsinki [WHO, 2001].Informed consent to all patients were obtained and agreed to use their data in this case report.

Author's contributions

First authors: DMR contributed to conceptualization, data curation, writing original draft, investigation, visualization, and writing a review, supervision, GM investigation, writing a review, and editing, RM writing review and data collection, AHM review, and editing, BK investigation, and editing. All authors read and approved the final manuscript.

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Knowledge and Practice of Breast Cancer Screening among Women in Enugu South, Nigeria

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Abstract- Globally, breast cancer has become a health priority due to its increasing incidence. Understanding the knowledge attitude and practice of breast cancer screening services is an essential step. The aim of this study is to determine the knowledge and practice of breast cancer screening among women in Enugu South. A descriptive cross sectional research design was adopted for the study. The study population for this study consisted of adult women aged 15 years at Enugu South LGA. A multistage random sampling technique was used in recruiting a total of 396 participants that participated in the study. A structured questionnaire was used for the study and Statistical Package for Social Science (SPSS) version 23.0 was used for the analysis of the study. Results from the study showed that 34.0% of the women were in the age range of 45-49 years. Good knowledge of breast cancer screening was observed in majority (85.6%) of the women. From the study 40.3% of the respondents had reportedly undergone breast cancer screening. The finding of the study revealed that the commonest factor affecting their practice of breast cancer screening was 'distance to facility' (19.3%).

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Knowledge and Practice of Breast Cancer Screening among Women in Enugu South, Nigeria

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Abstract- Globally, breast cancer has become a health priority due to its increasing incidence. Understanding the knowledge attitude and practice of breast cancer screening services is an essential step. The aim of this study is to determine the knowledge and practice of breast cancer screening among women in Enugu South. A descriptive cross sectional research design was adopted for the study. The study population for this study consisted of adult women aged 15 years at Enugu South LGA. A multistage random sampling technique was used in recruiting a total of 396 participants that participated in the study. A structured questionnaire was used for the study and Statistical Package for Social Science (SPSS) version 23.0 was used for the analysis of the study. Results from the study showed that 34.0% of the women were in the age range of 45-49 years. Good knowledge of breast cancer screening was observed in majority (85.6%) of the women. From the study 40.3% of the respondents had reportedly undergone breast cancer screening. The finding of the study revealed that the commonest factor affecting their practice of breast cancer screening was 'distance to facility' (19.3%). Considering the association between socio demographic characteristics and knowledge of breast cancer screening, parity (p =0.0008), age (p= 0.010), level of income (p=0.0092) and level of education (p=0.0327) were all associated. The study also showed that there was a statistically significant association between good knowledge and practice of breast cancer screening among women (p= 0.0032). The study concluded that women in Enugu south need to be encouraged to perform BCS regularly and earnestly report any abnormality to the health care providers since they generally showed willingness to participate if afforded an opportunity. The study recommended that policies must be implemented to accommodate low income earners and encourage breast cancer screening.

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I. Introduction

Globally in a publication by World Health Organization (WHO) (2016) breast cancer screening is an important practice in preventing breast cancer. Breast cancer is reported to be the second most common cancer in the world and, by far, the most frequent cancer among women with an estimated 1.67 million new cancer cases diagnosed in 2012 (25% of all cancers) (WHO, 2014). It ranked as the fifth cause of death from all cancers and the second most common cause of female cancer-related mortality worldwide (WHO; 2014; Abdel-Aziz *et al*., 2017; Diab *et al.,* 2018).

Studies have shown a reduction of breast cancer screening has a steady increase in the incidence of breast cancer in Nigeria from15.3per 100,000 in 1976 to 33.6 per 100,000 in 1992 to 52.1 per 100,000 in 2012 (Cancer Research, 2017; Olasehinde *et al.,* 2017; Hanson *et al.,* 2019; El Bcheraoui *et al.,* 2015). Globally, there is a regional variability in the incidence rates of this disease ranging from 27 per 100 000 in African and Middle Eastern countries to 96 per 100 000 in Western Europe and it is, also, the most frequent cause of cancer mortality among women in the less developed regions and the second in the developed countries (Aminisani *et al.,* 2016; National Cancer Institute, 2019; WHO, 2013). In developed countries, however, mortality from breast cancer has been on the decline despite the higher incidence of breast cancer. This is a result of early detection through organized screening programs and effective treatment modalities (Olasehinde *et al.,* 2017; Hanson *et al.,* 2019; El Bcheraoui *et al.,* 2015).

Breast cancer mortality has fallen considerably after the introduction of breast cancer screening in the western countries (WHO, 2013; Cancer Research, 2017). However, the screening is unavailable or less utilized (if available) in the developing countries where the majority of breast cancer deaths are occurred (Pierz et al., 2020; WHO, 2019; Keten et al., 2014; Aminisani *et al.,* 2016).

There is growing evidence that the knowledge of breast cancer screening is more aggressive in Nigeria than in the United States and Europe, including an

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earlier age of onset and a higher incidence of basal-like and HER2-enriched subtypes of the disease (Ojewole *et al.*, 2017; Sung *et al*., Rosenberg & Jemal, 2019; Keten *et al*., 2014). When detected early and treated promptly, these cancers have a high cure rate in a well-resourced high-functioning health system (Pruitt *et al.,* 2020). The aetiology of breast cancer is not well known (American Cancer Society, 2018). However, several risk factors have been shown to impact an individual's risk of developing breast cancer and their ultimate prognosis. These well-established risk factors include older age, family history, oral contraceptives, null parity, hormone replacement therapy, and early menarche, late first full-term pregnancy, late menopause, dense breast tissue, and tobacco smoking (Sung, Siege, Rosenberg & Jemal, 2019; Diab *et al.,* 2018). Breast cancer is curable when detected at an early stage. Women with early stage disease have an excellent prognosis with a 100% five years survival rate for stage 0 and I, while those with metastatic disease at diagnosis have a five years survival of around 20%, so it is important for women to be aware of the importance of early detection through screening(Diab *et al.,* 2018).

According to a report by Federal Ministry of Health in Nigeria (2015) early recognition and detection of Breast Cancer can play a significant role in reducing cancer morbidity and mortality as it gives more treatment options and increases survival rate if diagnosed early. Early detection of BC can be achieved by one of the following screening methods: breast self-examination (BSE), clinical breast examination (CBE), and mammography (Breast Cancer Now, 2019; Cancer Research, 2017; American Cancer Society, 2017). Although BSE alone is inadequate for early detection of BC, it is recommended by the American Cancer Society as an option for women starting from the early 20s of age as a method for breast awareness and early recognition and detection of BC. Unlike mammography and CBE, BSE does not require hospital visit and expertise, and it is cheap, simple, and non-invasive method that can performed by women themselves at home (Agodirin *et al.*, 2017). According to American Cancer Society (2018) recommendations, women should be aware how their breasts usually feel and report any breast changes without delay to their healthcare providers. Several previous studies have shown that female students had poor awareness and negative attitudes concerning BC and BSE. Such negative indicators continue to be present as a recent descriptive study among women in a community found that those women to have inadequate knowledge regarding BC and BSE (45.5%), fairly positive attitudes (56.3%), and low frequent practice of BSE (37.5%)(Ojewole *et al.*, 2017; Nwaneri *et al.,* 2017; Cancer Research, 2017; Denny *et al.*, 2012).

Worldwide, many interventional studies have been conducted to increase knwoeldge of BC screening and practice of BSE among women (Keten *et al.,* 2014; CDC, 2019; Anderson *et al*., 2018). For instance, a study by Abdel-Aziz *et al* (2017) evaluated the effectiveness of a breast health awareness program on knowledge of BC and BSE practice among women in Rural Nigeria based on the health belief model. The study revealed that the educational intervention had a positive impact on increasing BC knowledge among the participants. Similar findings were revealed among some young Nigerian women and Saudi women (Abdel-Aziz *et al.,* 2017). Therefore, all recommendations were to increase the level of the women's knowledge about BC and emphasize the importance of increasing BC awareness and promoting the practice of BSE for early detection of breast abnormalities (Hanson *et al.,* 2019; El Bcheraoui *et al.,* 2015; Agodirin *et al.,* 2017). Early detection and prompt attention as a result of adequate knowledge and awareness about breast cancer and screening methods go a long way in reducing the associated high mortality rate (Elobaid *et al.,* 2014; Aduayi *et al.,* 2016; Keten *et al.,* 2014).

Recent findings from a Nigerian Breast Cancer Study show that the majority of patients have advanced stage at diagnosis than has been reported in other populations (Arisegi *et al.,* 2019; Dodo *et al.,* 2016; Akande *et al.,* 2015). This underscores the need for systems-level interventions to downstage breast cancer in Nigeria (Dodo *et al.,* 2016; Akande *et al.,* 2015; Pruitt *et al.,* 2020). The causes of late-stage diagnosis are complex and, in addition to aggressive molecular subtypes, include lack of access to comprehensive screening and preventive care as well as social and cultural factors such as alternative healing, financial concerns, and lack of education (Breast Cancer Now, 2019; Pierz *et al.,* 2020; Akande *et al.,* 2015). Delayed diagnosis of breast cancer in Nigeria has been well documented and has a significant impact on breast cancer morbidity and mortality. Improved awareness campaigns and better understanding of the causes of delay in care is critical to develop relevant and effective screening measures. It is due to this that the current study aimed to investigate the knowledge and practice of breast cancer screening among women in Enugu South, Nigeria

II. Methods

a) Study Setting

Enugu South is a Local Government Area of Enugu State, Nigeria. Its headquarters are in the town of Uwani. It has an area of 67 km^2 and a population of 198,723 at the 2006 census. The postal code of the area is 400. The geographic coordinates of Enugu South is given as 5°57'40"N 8°42'39"E. The people of Enugu South are majorly farmers. Enugu South is a major producer of banana and plantain for the Nigerian market. It is known for the Christianity and Igbo

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speaking. The major occupations include agriculture and granite, quorite, and laterite mining and trading. The main agricultural food crops are cassava, yam, black beans, and cocoyam. The cash crops are palm nuts and cashew nuts. Pottery is another occupation.

b) Study Design

This study adopted a cross-sectional study using a quantitative method of data collection on the knowledge and practice of breast cancer screening among women at Enugu South.

c) Study Population

The study population for this study consisted of adult women aged 15 years at Enugu South LGA. The estimated population of women is 11,407.

d) Inclusion Criteria

This study includes;

i. All women aged 15 years and above at Enugu South who gave in their consent for the study.
ii. Any individual who volunteered to provide information vital to the research among women at Enugu South.

e) Exclusion Criteria

This study excludes;

i. Any woman aged 15 years and above at Enugu South who refuses to give in her consent for the study.
ii. Any woman aged 15 years and above at Enugu South who is sick, psychologically malnourished, disabled and on admission to the hospital during the time of data collection of the study.

f) Sample Size

The sample size for this study is 406 (see appendix A)

g) Sampling procedure

A multistage random sampling technique was used. The procedure was as follows: Stage 1: *Selection of Communities*; Simple random sampling was used to select 5 Communities from the total number of communities in Enugu South LGA. Stage 2: Selection of Villages: Two villages each were selected from each of the five selected communities. Also Systematic random sampling was once more is used to select households on each street to give every household an equal chance of selection. This would be done by the researcher. Finally, simple random sampling was used to select 3 females of reproductive age (15years and above) in each household giving a total of 406 respondents.

h) Instrument for Data Collection

A self administered semi structured questionnaire was used for the study on the knowledge and practice of breast cancer screening among women at Enugu South. The questionnaire was designed for simplicity and assimilation by the respondents.

i) Validity of the Instrument

The research instrument being the questionnaire which was used for data collection was developed by researcher and submitted to the project supervisor as well as two experts from department of public health for face validity and proper scrutiny in order to ensure that the questionnaire met the objectives of study.

j) Reliability of Instrument

Reliability of the instrument was determined using test retest method. Copies of the questionnaire were given to some women outside the area of study by the researcher because this area for reliability testing shared similar characteristics with Enugu South LGA that was used for the study. Chrombach alpha test was used to test for the reliability coefficient of the questionnaire.

k) Method of Data Collection

Data was obtained using a self administered based semi structured questionnaire. This was done with the aid of Two (2) field assistants who were Hired and trained to aid the researcher in the data collection process. The purpose of the research was explained face to face to the respondents before distribution of the questionnaires to them.

l) Method of Data Analysis

The Statistical Package for the Social Sciences (SPSS) was used in the analysis of the data gotten from the study. Results were expressed in percentages, frequencies, tables and charts (Descriptive Statistics). Chi square test was then used to analyze the hypothesis of the study p = (0.05).

m) Ethical Consideration

A letter of introduction and ethical clearance was obtained from the Department of Nursing Sciences, University of Nigeria Nsukka before the research was conducted. The purpose of the research was explained to each respondent and verbal informed consent obtained from them before inclusion into the study. Also, anonymity of the respondents was also assured and ensured. The confidentiality of the information they gave was also be maintained.

III. Results

A total of Four hundred and six (406) copies of questionnaires were distributed for the study and three hundred and ninety-six (396) questionnaires were retrieved and they were properly filled and crosschecked for correctness and were used for the purpose of the analysis.

a) Socio-demographic Characteristics

From table 1 below, it was posited that 34.0% (135) of the women represented age groups between 45-49, 30.3% (120) of the women were 50 years and

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above, 21.4% (85) of the respondents were 35-44 years of age, 8.2% (32) were aged 25-34, and 6.1% (24) aged 15-24 years. 63.2% (250) of the women were of Igbo origin, 29.1% (115) reported 'others', 5.1% (20) Yoruba, and 2.7% (11) Hausa/Fulani. 66.9% (265) of the respondents were Christians, 14.7% (58) listed religions not included in the options but label 'others', 11.4% (45) Traditional and 6.9% (28) Muslim. 35.9% (142) of the women had a child, 8.9% (35) had two children, 26.6% (105) had 3 children and above, and 28.7% (113) had no children. Concerning the education level of the respondents, 39.0% (154) had attained tertiary education, 28.9% (115) for secondary education levels, 22.1% (88) had attained primary education levels and just 9.9% (39) had informal education levels. Students among the respondents totaled 24.7% (98), 25.2% (100) were civil servants, 23.9% (95) 'farmers', 5.9% (23) identified as traders, and 20.3% (80) 'others'. 35.8% (142) reported 'yes' concerning monthly income satisfaction, while 64.2% (254) of the women said "no". 42.8% (169) of the respondents were single, 35.2% (139) married, 16.5% (65) separated, and 5.6% (22) widowed. When the women were asked about their household level of income, 16.5% (65) reported income above 100,000, 20.9% (83) between 2,000-10,000, 10.2% (40) earned from 11,000-30,000, 2.9% (11) 1-1,000, 19.9% (79) listed 'other' income levels, 18.6% (74) earned figures from 61,000-100,000, and 11.0% (44) from 31,000-60,000. 47.4% (188) of the respondents affirmed they had a health plan at a healthcare center, while 52.6% (208) reported they did not.

Table 1: Socio Demographic Characteristics of the Women

Characteristics	Frequency (n=396)	Percentage (%)
Age		
15-24	24	6.1
25-34	32	8.2
35-44	85	21.4
45-49	135	34.0
50 and Above	120	30.3
Total	**396**	**100**
Ethnicity		
Igbo	250	63.2
Hausa/Fulani	20	5.1
Yoruba	11	2.7
Others	115	29.1
Total	**396**	**100**
Religion		
Christianity	265	66.9
Muslim	28	6.9
Traditional	45	11.4
Others	58	14.7
Total	**396**	**100**
Number of Children (Parity)		
None	113	28.7
1	142	35.9
2	35	8.9
3 and above	105	26.6
Total	**396**	**100**
Education level		
Informal education	39	9.9
Primary	88	22.1
Secondary	115	28.9
Tertiary	154	39.0
Total	**396**	**100**
Occupation		
Student	98	24.7
Farmer	95	23.9
Trader	23	5.9
Civil servant	100	25.2
Others	80	20.3
Total	**396**	**100**

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Marital Status		
Married	65	16.5
Single	169	42.8
Separated	22	5.6
Widowed	139	35.2
Total	**396**	**100**
Household level of Income		
1-1,000	83	20.9
2,000-10,000	11	2.9
11,000-30,000	40	10.2
31,000-60,000	44	11.0
61,000-100,000	74	18.6
Above 100,000	65	16.5
Others	79	19.9
Total	**396**	**100**
Are you satisfied with your monthly income?		
Yes	142	35.8
No	254	64.2
Total	**396**	**100**
Do you have a Health plan at any healthcare Center?		
Yes	188	47.4
No	208	52.6
Total	**396**	**100**

b) Knowledge of Breast cancer screening among Women

From table 2 below, majority of the respondents 85.6% (339) demonstrated knowledge of breast cancer screening, while 14.4% (57) denied. For example (see figure 1. below), 23.0% (87) of the respondents reported 'newspaper/magazines' as their sources of information on breast cancer screening, 19.0% (75) said "Tv/radio programs", 18.9% (75) reported social media, 3.8% (15) health practitioners, 14.9% (59) parents/family, 11.4% (45) school, and 9.0% (36) reported sources not listed but label 'others'. 40.3% (159) of the women affirmed they had been part of a breast cancer screening, 32.5% (129) reported 'no', and 27.3% (108) were not sure. Respondents who accepted to have undergone breast cancer screening reported to have done so between 6 months to a year (26.4%), 24.4% (39) reported 'longer than a year', 21.9% (35) said "4-6 months", 14.8% (23) reported 2-3 months, and 12.6% (20) reported in less than a month. 52.5% (208) of the women affirmed that mammography is a method used to screen for breast cancer, while 47.5% (188) replied "no". Women in Enugu-South accepted that breast self examination is encouraged as part of a breast education program, while 15.3% (60) said "no". When the respondents were asked if lump swelling under armpit, bleeding or discharge and nipple retraction is a warning sign of breast cancer, majority agreed (78.8%), while 21.2% (84) reported otherwise.

Table 2: Knowledge of Breast cancer screening among Women

Variables	Frequency (n=396)	Percentage (%)
Have you heard about Breast cancer screening?		
Yes	339	85.6
No	57	14.4
Total	**396**	**100**
What is your source of information?		
School	45	11.4
Parents/Family	59	14.9
Social Media	75	18.9
TV/Radio programs	75	19.0
Health Practitioners	15	3.8
Newspaper/Magazines	91	23.0

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Others	36	9.0
Total	**366**	**100**
Have you been part of a Breast Cancer Screening Program		
Yes	159	40.3
No	129	32.5
Not sure	108	27.3
Total	**396**	**100**
If Yes, when was that?		
Not Yet	0	0
less than a month	20	12.6
2-3 months	23	14.8
4-6 months	35	21.9
6 months to a year	42	26.4
longer than a year	39	24.4
Total	**159**	**100**
Mammography is a method used to screen for breast cancer		
Yes	208	52.5
No	188	47.5
Total	**396**	**100**
Breast self examination is encouraged as part of a breast education program		
Yes	336	84.7
No	60	15.3
Total	**396**	**100**
Lump swelling under armpit, bleeding or discharge and nipple retraction is a warning sign of breast cancer		
Yes	312	78.8
No	84	21.2
Total	**396**	**100**

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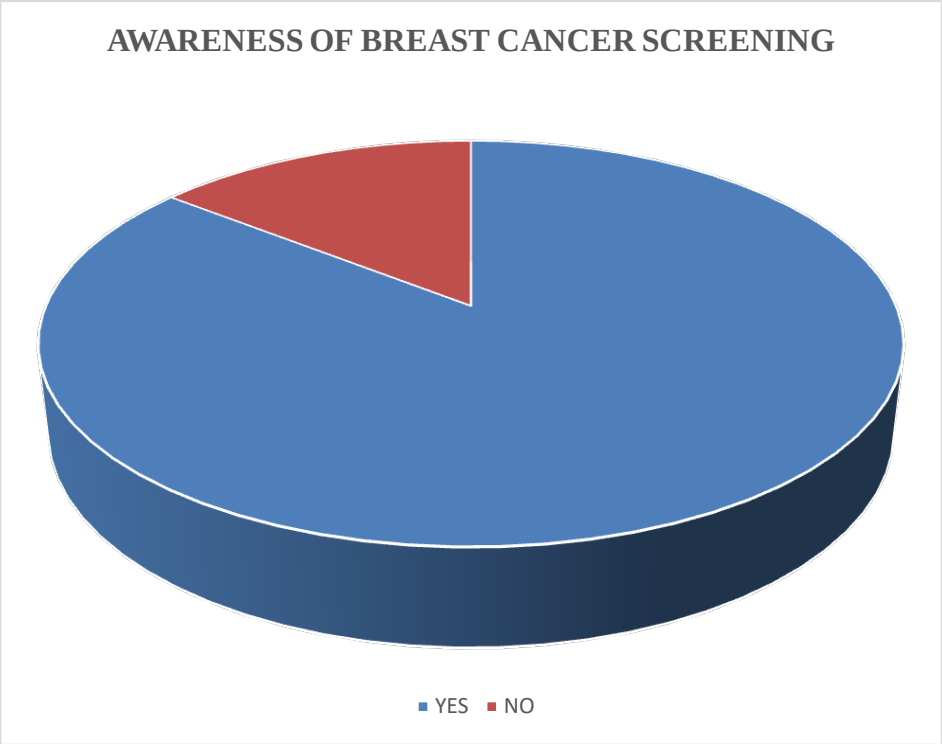

Fig.1: Awareness of Breast Cancer Screening

c) *Practices of Breast Cancer Screening among Women*

From table 3 below, majority of the respondents reportedly demonstrated their approval to undergo breast cancer screening if offered a chance (92.5%), while 7.5% (30) denied. 53.9% (214) of the respondents reported 'Yes" when they were asked if they had been advised by a physician to screen the breast prior to the time of this investigation, 26.4% (105) could not remember, and 19.7% (78) said "No". 47.0% (186) of the respondents had not screened for breast cancer or any infection relating to the breast before filling the questionnaire, 36.3% (144) replied "Yes", and 16.7% (66) reportedly could not remember. 32.7% (47) of the respondents who reported 'yes' said they last screened for periods longer than a year, 24.6% (35) reported 4-6 months ago, 18.1% (26) said "6 months to a year", 16.6% (24) reported 'in less than a month', and 8.0% (12) reported 2-3 months ago. When they were asked concerning reasons for breast cancer screening, over half of the women (57.3%) reported 'for prevention', 18.1% (26) explained that they were presented with symptoms, 16.6% (24) just decided to go for the examination, and 8.0% (12) as a result of cases in the family respectively. 85.4% (338) of the women had never had an abnormal test result in breast cancer screening, while 14.4% (58) reported 'Yes'.

Table 3: Practices of Breast Cancer Screening among Women

Variable	Frequency (n=396)	Percentage (%)
Do you Practice breast cancer screening if offered a chance?		
Yes	366	92.5
No	30	7.5
Total	**396**	**100**
Have any physician advised you to screen the breast before?		
Yes	214	53.9
No	78	19.7
Cannot Remember	105	26.4
Total	**396**	**100**

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Have you screened for breast cancer or any infection relating to the breast before?		
Yes	144	36.3
No	186	47.0
Cannot Remember	66	16.7
Total	**396**	**100**
If YES when was that?		
less than a month	24	16.6
2-3 months	12	8.0
4-6 months	35	24.6
6 months to a year	26	18.1
longer than a year	47	32.7
Total	**144**	**100**
What was your reason for the breast cancer screening?		
Presented with symptoms	26	18.1
Cases in the family	12	8.0
For prevention	82	57.3
Just decided to go for the examination	24	16.6
Total	**46**	**100**
Have you ever had abnormal test result in Breast cancer screening?		
Yes	58	14.6
No	338	85.4
Total	**46**	**100**

d) Factors Influencing the Practice of Breast Cancer Screening among Women

Table 4 below demonstrated the factors affecting the practice of breast cancer screening practice among women in the study. 19.3% (82) reported distance to facility, 18.7% (79) said "family/husband acceptance", 13.8% (58) reported lack of information, 13.3% (56) financial constraints, 11.3% (48) behavior of health workers, 10.0% (42) cultural related factors, and 0.5% (2) said "religious factors".

Table 4: Factors Influencing the Practice of Breast Cancer Screening among Women

Variable	Frequency (n=396)	Percentage (%)
Which of the following related as possible factors affecting your Utility of Breast cancer screening		
Distance to facility	82	19.3
Cultural related factors	42	10.0
Family/Husband Acceptance	79	18.7
Financial Constraints	56	13.3
Lack of Information	58	13.8
Religious Factors	2	0.5
Behavior of Health workers	48	11.3

e) Association between the knowledge of Breast cancer screening and the Socio demographic characteristics of females

Table 5 below showed the results for the test of a statistically significant association between socio-demographic characteristics and knowledge of breast cancer screening among women in Enugu South Local Government Area, Enugu State. There was a statistically significant association between age of women and knowledge of breast cancer screening (p= 0.010). Given the association between marital status of women and knowledge of breast cancer screening among women in the study population (p=0.300), there was no significant association. On the hypothesis between number of children (parity) and knowledge of breast cancer screening among women in primal population, There was also a statistically significant association (p=0.0008). Given the association between level of income of women and knowledge of breast cancer screening in the study population, there was a statistically significant association (p=0.0092). There was a statistically significant association between level of education and knowledge of breast cancer screening in the study population (p=0.0327). Finally, there was no statistically significant association between occupation and knowledge of breast cancer screening in the study population (p=0.127).

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Table 5: Association between the knowledge of Breast cancer screening and the Socio demographic characteristics of females

Socio Demographics	Knowledge of Breast cancer screening		X2	P-value	Decision
	Yes (%)	No (%)			
Age	84.8%	15.2%	12	0.010	S
Marital Status	53.7%	46.3%	2.0	0.300	NS
Number of Children (Parity)	69.7%	29.3%	3.332	0.0008	S
Level of income	95.8%	4.2%	8.57	0.0092	S
Level of Education	70.1%	29.9%	17	0.0327	S
Occupation	59.5%	40.5%	1.97	0.127	NS

f) Association between the knowledge of Breast cancer screening and the Practice of breast cancer screening among women at Enugu South

Table 6 below showed the results for the test of a statistically significant association between knowledge of breast cancer screening and practice of breast cancer screening among women. There was a statistically significant association between good knowledge and practice of breast cancer screening among women (p= 0.0032).

Table 6: Association between the knowledge of Breast cancer screening and the Practice of breast cancer screening among women at Enugu South

Practice of breast cancer screening	knowledge of breast cancer screening		X2	P-value	Decision
	Good Knowledge (%)	Poor Knowledge (%)			
Yes	89.0%	11.0%	1.9376	0.0032	*Sig*
No	32.8%	67.2%			

IV. Discussion

Findings of this study respect to the socio demographic characteristics of the respondents, 34.0% of the women were in the age range of 45-49 years and this is comparable to findings of Ogunkorode *et al.* (2017) which showed that 35% of the population studied was between the ages of 45-49 years. Also, observation from this study showed that majority of the participants in the breast cancer screening survey were Christians (66.9%) and of Igbo origin (63.2%). This is justified given the study was conducted in the south-eastern part of Nigeria predominated by people of Igbo and Christian origin. A similar study conducted in the South-eastern region of Nigeria suggested similar findings regarding the predominance of Christians and Igbo people (Azubuike *et al*., 2018).

Good knowledge of breast cancer screening was observed in majority (85.6%) of the women. The high level of knowledge was also observed in studies according to Ogunkorode *et al.* (2017) and Okoronkwo *et al.* (2015) where majority, of the respondents had good knowledge about breast cancer. However this as in contrast to the level of knowledge reported among students in Turkey where low knowledge level was reported (Hanson *et al.,* 2019). In the study, 23.0% of the women listed newspaper/magazines as their source of information on breast cancer screening. This could be due to some campaigns and awareness on breast cancer screening on mainstream media. Additionally, 40.3% of the respondents had reportedly undergone breast cancer screening. This is however in contrast with a study by Aminisani *et al.* (2016) conducted in Uganda. Another study conducted by Bcheraoui *et al.* (2015) suggested lower figures concerning participation in breast cancer screening. From this study, it was revealed that 52.5% of the women affirmed that mammography is a method used to screen for breast cancer and 78.8% correctly reported that lump swelling under armpit, bleeding or discharge and nipple retraction is a warning sign of breast cancer. A publication by Diab *et al.* (2018) revealed figures which

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corroborate this finding and this is also in agreement with the finding of Akande *et al*. (2015) where majority of the students were well informed about mammography as a screening method for breast cancer. This was also observed by Kamińska *et al.* (2015) and finding is similar to the results of another study conducted. From the study 92.5% of the women demonstrated their approval to utilize breast screening services if offered a chance these points out the lack of access towards breast cancer screening among respondents. A previous study by Diab *et al.* (2018) suggested similar findings among respondents in a Kenyan study. 53.9% of the women accepted they had been advised by a physician to screen the breast prior to the time of this investigation. A study by Poehls (2019) corroborates this finding and demonstrated that physicians actively sensitized their female patients on breast cancer screening. This study revealed that 85.4% of the women had never had an abnormal test result in Breast cancer screening as supported by several studies (Kanaga *et al.,* 2011; Karabay *et al.* 2018; Al-Hussami, 2014).

The finding of the study revealed that the commonest factor affecting their practice of breast cancer screening was 'distance to facility' (19.3%). This goes in consistence with a study by Poehls (2019) on the practice of breast cancer screening screenings. Another study by *Hedge et al. (2018)* in agreement to this finding and suggests that 26.6% of women who underwent breast cancer screenings listed affecting factors such as financial constraints, followed by distance to facility.

Findings from this study regarding the association between Socio-demographic characteristics and practice of Breast cancer screening among women revealed that Age is significantly associated with practice of breast cancer screening screening among women($p = 0.010$). Study shows that older women groups utilized breast cancer screening relative to younger groups. This goes in line with a study by *Hedge et al. (2018)* which found age to be associated with practice of breast cancer screening ($p = 0.00271$). Further investigation into the study demonstrated that marital status is not significantly associated with the practice of breast cancer screening ($p = 0.300$). This goes in line with a report published by Al-Amri (2015) that there was no significant association. This implies that women who wanted to utilize screenings did, irrespective of their marital status. Although certain studies suggested some women did not participate in screening exercises due to permission/acceptance from their husbands (Chigbu *et al.*, 2017; Nnebue *et al.*, 2018). Also, from the study among women in Enugu South, it was posited that there was a significant association between number of children (Parity) and practice of breast cancer screening among women in the study population ($p =0.0008$). Few studies support this finding (Adejumo *et al*., 2018; Chigbu *et al.*, 2017; Nnebue *et al.*, 2018; Olasehinde *et al.*, 2017). Considering the hypothesis between level of income of women and practice of breast cancer screening, there a significant association ($p =0.0092$). This goes in consistence to a previous study by Al-Amri (2015). This signifies that women with better level of income were more likely to utilize breast cancer screening services. This study also indicates that women with higher level of education were significantly involved in breast cancer screening than those with low levels of education. Women without any formal education level hardly came in for screening. This indicates that more enlightened a person is, the more likely they were to undertake breast cancer screenings. Hence level of education of women and practice of breast cancer screening are significantly associated ($p = 0.0327$). A preceding study by Al-Amri (2015) confirms this finding. Findings of this study showed an association between knowledge of breast cancer screening and practice of breast cancer screening among female women($p= 0.00532$).This implies that women who were well informed know the importance and would easily seek breast cancer screening as opposed to those who lacked information. A study by Poehls (2019) corroborates this finding on the association between knowledge of breast cancer screening and practice of breast cancer screening.

V. Conclusion

Breast cancer is a major health concern and remains the most common malignancy in women worldwide. In this study, It was seen that age, educational level, level of income, marital status and knowledge were all related with practice of breast cancer screening among the women in Enugu South. Findings from this study establish that even though a number of women showed considerable knowledge of breast cancer screening, several others were deficient of relevant information. Women need to be encouraged to perform BCS regularly and earnestly report any abnormality to the health care providers since they generally showed willingness to participate if afforded an opportunity. Also, perceived factors affecting breast cancer screening practices such as distance to facilities must be put into consideration to ease uptake. Emphasis must be made on the importance and effectiveness of breast cancer screening. Also Policies must be implemented to accommodate low income earners and encourage breast cancer screening.

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Appendix A

Sample size determination

The sample size will be determined using the Yamene formula (1967) for sample size determination.

$$n = \frac{N}{1+Ne^2}$$

Where:
n is the desired sample size
N is the population size (11,407)
e is margin of error (0.05)
Therefore,

$$n = \frac{N}{1+Ne^2}$$

$$\frac{11,407}{1+11407* (0.05)^2}$$

$$\frac{11,407}{29.5175}$$

n = 386.448 = 386

Furthermore, to account for 5% Non Response Rate, (i.e. 95% response rate)

n = n/expected response rate

$$\frac{386}{0.95}$$

n =406.3157…….. 406.
i.e the total sample size for the study would be **406.**

Appendix B

Questionnaire on the Knowledge and Practice of Breast Cancer Screening among Women in Enugu South, Enugu, Nigeria

SECTION A: SOCIO DEMOGRAPHIC CHARACTERISTICS OF WOMEN

INSTRUCTION: Please tick (√) the correct options besides each question and also fill in the spaces provided where appropriate with the correct options.

1. What is your age range? (a) 15-24 [ ] (b) 25-34 [ ] (c) 35-44 [ ] (d) 45-49 [ ] (e) above 50 [ ]
2. What is your Religion? (a) Christianity [ ] (b) Muslim [ ] (c) Traditional [ ] (d) Others **(Please Specify)** _____
3. Ethnicity (a) Igbo [ ] (b) Hausa [ ] (c) Yoruba [ ] (d) Fulani [ ] (e) Others **(please specify)**_____
4. Marital status (a) Married [ ] (b) Single [ ] (c) Separated [ ] (d) Widowed [ ]
5. Education level (a) No formal education [ ] (b) Primary [ ] (c) Secondary [ ] (d) Tertiary [ ]

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6. Your occupation: (a) Artisan e.g Carpenter, Hairdresser, Tailor, Driver [ ] (b) Civil servant e.g Teacher [ ] (c) Self-employed e.g Trader, Photographer [ ] (d) Unemployed [ ] (e) Professionals e.g. Doctor, Nurse, Lawyer, Accountant [ ] (f) Others (please specify)_____
7. What is your Level of Income (a.) 1-1,000 [ ] (b.) 2,000-10,000 [ ] (c.) 11,000-30,000 [ ] (d.) 31,000-60,000 [ ] (e) 61,000-100,000 [ ] (f) above 100,000 [ ] (g) others (specify)……
8. Number of children (Parity) (a.) None [ ] (b.) 1 [ ] (c.) 2 [ ] (d.) 3 [ ] (e) 4 and above [ ]
9. Are you satisfied with your monthly income? (a) Yes [ ] (b) No [ ] (c) Somehow [ ]
10. Do you have a Health plan at any healthcare Center? (a) Yes [ ] (b) No [ ]

SECTION B: KNOWLEDGE OF BREAST CANCER SCREENING AMONG WOMEN

INSTRUCTION: Please tick (√) the correct options besides each question and also fill in the spaces provided where appropriate with the correct options.

11. Have you heard about Breast Cancer Screening? (a) Yes [ ] (b) No [ ]
12. What is your source of information on breast cancer Screening? (a) School [ ] (b) Parents/family [ ] (c) Social media [ ] (d) Tv/radio programs [ ] (e) Health practitioner i.e nurse, doctor, auxiliary health personnel etc. [ ] (f) news papers/ magazines [ ] (l) others(*please specify)*_____
13. Have you been part of a Breast Cancer Screening Program (a) Yes [ ] (b) No [ ] (c) Not Sure [ ]
14. When was that? (a) Not Yet [ ] (b) less than a month [ ] (c) 2-3 months [ ] (d) 4-6 months [ ] (e) 6 months to a year [ ] (f) longer than a year[ ]
15. Mammography is a method used to screen for breast cancer (a) Yes [ ] (b) No [ ]
16. Breast self examination is encouraged as part of a breast education program (a) Yes [ ] (b) No [ ]
17. Lump swelling under armpit, bleeding or discharge and nipple retraction is a warning sign of breast cancer (a) Yes [ ] (b) No [ ]

SECTION C: PRACTICES OF BREAST CANCER SCREENING AMONG WOMEN

18. Do you Practice breast cancer screening if offered a chance? a) Yes [ ] (b) No [ ]
19. If No to **Question (25)** Why? _____
20. Have any physician advised you to screen the breast before? (a) Yes [ ] (b) No [ ] (c) Cannot Remember
21. Have you screened for breast cancer or any infection relating to the breast before? (a) Yes [ ] (b) No [ ] (c) Cannot Remember
22. If **YES** when was that? (a) less than a month [ ] (b) 2-3 months [ ] (c) 4-6 months [ ] (d) 6 months to a year [ ] (e) longer than a year[ ]
23. What was your reason for the breast cancer screening? (a) Presented with symptoms [ ] (b) Cancer cases in the family [ ] (c) for prevention [ ] (d) Just decided to go for the examination [ ] (f) Others *(please specify)*_____
24. Have you ever had abnormal test result in breast cancer screening? (a) Yes [ ] (b) No [ ]

SECTION E: FACTORS INFLUENCING THE PRACTICE OF BREAST CANCER SCREENING AMONG WOMEN

Please tick (√) the correct options that related as possible factors influencing yourpractice of breast cancer screening in the spaces provided in the Table below.

S/N	Factors	Yes	No
1.	Family/Husband Acceptance		
2.	Cultural related factors		
3.	Distance to facility		
4.	Financial Constraints		
5.	Lack of Information		
6.	Religious Factors		
7.	Behavior of Health workers		

Thank You

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Genetic Profile of Human Papilloma Virus Circulating in Cervical Precancerous-Lesions of Cameroonian Women

By Embolo Enyegue Elisée Libert, Ananga Noah Sidonie, Awalou Halidou, Halmata Mohamadou, Ndipho Tatou Christian Kitchener, Doh Ndeh Gilbert, Banai Thomas, Bell Eric Michel, Essame Oyono Jean Louis & Koanga Mogtomo Martin Luther

Abstract- *Background:* Cervical cancer is the most common cancer after breast cancer worldwide, especially in developing countries. Infection by Human Papillomaviruses (HPV), In Cameroon, there is a paucity of studies focused on its epidemiology. This study aimed at appraising the diversity of HPV genotypes circulating in precancerous cervical lesions observed in Cameroonian women. For this study, a total of 4063 women fulfilled inclusion criteria and they were enrolled. For this sample, only 1947 women presented precancerous cervical lesions. This cross sectional study carried out in three areas of the Cameroon namely Niete, Mokolo and Yaounde.

Methods: Cervical swabs were obtained from each participant and thereafter undergone cytological analysis relied on Pap test techniques. DNA was extracted from positive smears for genotyping of Human papilloma virus using multiplex PCR (polymerase chain reaction) method. Data analysis was done by GraphPad Prism 5 and XLSTAT.

Keywords: *human papilloma virus, molecular epidemiology, precancerous lesions; cervical cancer; cameroon.*

GJMR-F Classification: *WP 840, QZ 20.5*

Strictly as per the compliance and regulations of:

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Results: Molecular analysis results obtained, demonstrated that around 75% (1460) of the women with precancerous cervical lesions in the study population presented six (6) different Low-Risk (LR) (HPV 6, HPV 61, HPV 11, HPV 81, HPV 62 and HPV 70) and five (5) different High-Risk (HR) (HPV 45, HPV18, HPV16, HPV16, HPV35 and HPV84) genotypes of HPV (P value < 0.001). In several cases, combinations of genotypes of High-Risk HPV and Low-Risk HPV were detected. However, the highest rate of LSIL (80.8%) was observed in women with genotype 35.

Conclusions: Thus, it is possible to confirm with confidence that, there is genotypic diversity of HPV among Cameroonian women with precancerous cervical lesions.

Keywords: human papilloma virus, molecular epidemiology, precancerous lesions; cervical cancer; cameroon.

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I. Introduction

Though cervical cancer (CC) is largely preventable, it is still the second most common female cancer internationally and the leading cause of cancer deaths among females in African countries[1]. Despite the considerable success of research results based on visual inspection and cytological analyses, cervical cancer still remains a public health problem in Cameroon. Cervical precancerous lesions are often correlated to Human Papilloma Virus infections[2], human papillomavirus (HPV) is currently the most common pathogen responsible for female cancers[3]. Indeed, HPV are viruses that belong to the Papillomaviridae family, consisting of an icosahedral capsid of 55 nm in diameter and a double-stranded DNA of 8,000 base pairs[2]. HPVs have exclusive tropism for metaplastic cells in the junctions of squamous and glandular epithelia and elicit cytopathogenic effects with transformation of keratinocytes into koilocytes [4]. In Cameroon, very few people work on HPV, so very few studies have been conducted. No data are clear concerning viral genotypes circulating in this country and there is no information on risk factors available. In 2009, a US research team headed by Desruisseau, demonstrated that; oncogenic HPV subtypes 45 and 58 were more prevalent in Cameroon [4]. Several other studies conducted in Africa by other researchers had presented different dominant genotypes. In Ethiopia for example, Human papilloma virus type 16 was the most prevalent genotype identified from the subjects screened[5]. In Angola, The most prevalent HPV genotypes were HPV16, HPV6 and HPV 18[6]. Morocco data presented, high rates of infection with HPV genotypes in sexually active Moroccan women making molecular investigation for HPV16, 18 and 31 essential in clinical approach. However, HPV 33, 35 and 45 are less frequent in this population[7], [8]. In Mozambique, among women with cervical cancer HPVs 16 and HPV 18 were the two most frequently identified genotypes (47.0% and 31.3%, respectively), followed by HPV Types 51, 52, 45, 35, 33 and 31[9]. Studies in south Africa demonstrated high prevalence of HPV and multiple HPV

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infection among HIV-positive women compared to HIV-negative women across all ages [10]. Study carry out in Zambia shows that among high-risk (HR) types, HPV 52 (37.2%), 58 (24.1%) and 53 (20.7%) were more common overall than HPV 16 (17.2%) and 18 (13.1%) in women with high-grade squamous intraepithelial lesions or squamous cell carcinoma (SCC) on cytology[11]. All this Human Papilloma Viruses diversity demonstrates the importance of accurately determining HPV genotypes and subtypes that prevail in the sub-Saharan zone, especially in Cameroon.

II. Material and Methods

a) Study sites

This was a cross-sectional study, which took place in hospitals in the selected areas of three region of Cameroon (the South, The Far North and the central regions).

a. *District of Niete*

Niete is an agro-industrial locality located in southern Cameroon near the Atlantic coast in the Ocean Division and the Southern Region. The commune of Niete has about 40 894 inhabitants in 28 villages. The communal population is composed of 19 137 men, 11 154 women, 5 655 and 4 948 young people aged 5- 16 years and less than 5 years respectively. The work was carried out in three subdivision medical centers, namely the V15 hospital, the V4 hospital and the ADJAP hospital.

b. *District of Mokolo*

Mokolo is a city located in the Far-North region, near the border with Nigeria. It is the county town of the Mayo-Tsanaga Division. The study was conducted at the District Hospital of Mokolo. The commune of Mokolo is one of the largest municipalities in the Far North with an area of 1650 km^2 for a population estimated at 310,000 inhabitants in 106 villages. The economy of Mokolo is relied mainly on agriculture (rainy season sorghum, dry season sorghum, groundnut, cowpea, soybean, sweet potato, and vegetable and fruit crops), livestock (cattle, goats, sheep, and poultry), small trade and crafts.

c. *District of Yaoundé 1*

The district of Yaounde III, Department of Mfoundi, Central Region. It covers an area of 5552 hectares for a population estimated at 281 586 inhabitants, ie a density of about 507habitants / km2. Gender related statistics estimate 141,525 for males and 140,011 for females; Leading to a sex ratio of 101.05%. It is managed by a municipal council of 41 members, a communal executive composed of a mayor and four deputies. There are several hospitals and dispensaries, where we collected the data.

b) Study population and sample size

These analyses targeted all women with or without cancer at different stages of development.

i. *Selection criteria*

a. *Inclusion*

All Cameroonian women over the age of 18 were eligible. They should not have undergone hysterectomy, they had to be willing to participate in the study, the must have signed the informed consent form, they must be sexually active.

b. Exclusion

All pregnant women;
All women with cervical cancer.

ii. *Sampling method*

A convenient sampling technique in which potential participants were consecutively recruited at the different sites.

iii. *Questionnaire*

An investigator administered semi structured questionnaire was used to collect data of each woman through 15-20 minutes' individual interview. The first part of questionnaire focused on sociodemographic information such as age, level of occupation or religion. The second part allowed for collecting obstetric information as well as those related to sex behavior.

iv. *Cervix sample collection and visual inspection*

After counseling participants were placed in gynecological position on an examination table. A clean, sterile non-lubricated speculum was gradually introduced into their vagina for eye examination of cervix. 2 Samples were obtained by collecting exfoliated cells, from the transformation zone of the cervix using cytobrush and ayre spatula. Thereafter, these cells from ayre spatula were transferred directly onto a slide and fixed using the conventional technique and the cells from cytobrush were used for PCR. The visual inspection with acetic acid was performed according the atlas of cytology, with Lugol's iodine recommendations. Results were classified as: normal cervix, abnormal cervix, and cervix with suspected cancer. The cytological analysis has been performed using the Papanicolau test, and the Bethesda classification system for interpretation of results has been used.

v. *Cytological analysis*

Pap smears were obtained by collecting exfoliated from the transformation zone of the cervix using ayre spatula. The cells were transferred directly to a slide and fixed using the conventional technique. The Bethesda system was used to interpret results from slides.

c) DNA extraction

The cells collected with cytobrush were used for virus isolation.DNA extraction of fresh cervical cells was made using the QIAGEN extraction kit, which is a

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commercially available. Extraction was made according to the manufacturer.

d) HPV genotyping strategy

a. *Primers design*

The table in supplementary material shows the newly designed primers that were used throughout this study.

The beta globin gene was used to check the quality of the reaction.

DNA sequences of the HPV genotypes targeted obtained from Genbank. The primers were modeled for each type of HPV which the literature showed a high prevalence in Cameroon, as presented above. With the Primer 3 online program (https://primer3.ut.ee/), we were able to generate the primers for 6 low risk genotypes (LR), (6, 11, 61, 62, 70 and 81) and 6 high risk genotypes (HR) (16, 18, 35, 45, 58 and 84)

b. *Mix and PCR*

The master mix contains pre-optimized concentrations of HotStarTaq DNA Polymerase and MgCl2, plus dNTP, and a PCR buffer that contains the new MP factor. The use of a master-mix format reduces the time and handling for the reaction configuration and increases the reproducibility by eliminating many possibilities.

The following conditions were used for PCR amplification on a Thermal Cycler (Applied Biosystems): Denaturation for 3 minutes at 91°C, followed by 42 cycles of 27 seconds at 94°C, 45 seconds at 50°C, and 10 minutes at 64°C, and a final elongation step of 5 minutes at 65°C. After amplification, the reaction mixture was transfer for electrophoresis to 8°C.

Analyzing of the PCR product was done using agarose gel electrophoresis, which separates DNA products on the basis of size and charge, it allows for the determination of the presence and the size of the PCR product. Visualization was done using transilluminator.

c. *Control quality*

The β-globin was a quality control gene for PCR amplification used to show the smooth progress of the PCR. The β-globin (-) represents the samples whose β-globin internal control gene could not be amplified. In our study, 5.16% of the samples were β-globin (-); and as a result were they were declared unsatisfactory. β-globin (+) represents samples whose β-globin internal control gene has been successfully amplified which accounted for 94.84%.

e) Ethical statement

The study was carried out in conformance with the guidelines for human experimental models in clinical research as stated by the Cameroon Ministry of Public Health and the Helsinki declaration. To do so, ethical clearance was issued by the National Ethics Committee of Cameroun with registration number 2014/08/485/CE/CNERSH/SP. likewise, administrative clearance was issued by regional delegations. The aim and objectives of the study were explained to each woman in the language they understood best (English or French), and their questions were answered. Only women who signed an informed consent form for their participation were enrolled. Participation in the study was strictly voluntary and women were free to decline answering any question or totally withdraw if they so wished at any time.

f) Statistical analysis

Data were keyed and verified for consistency into Excel spreadsheet and thereafter analyzed with Graph Pad Prism version 6. Independence Chi-square test and one-way ANOVA, were used to compare results. Qualitative variables were presented as percentage with confidence interval at 95% in tables and graphics. XLSTAT 2015 software was used to perform principal component analysis (PCA) in order to establish correlations between ≥ 2 quantitative variables. Significance was set at $P \leq 0.05$.

III. Results

a) Characterization of sample collected

The table 2 below presents some patients with atypical profiles who were selected for molecular analysis and cytology and PCR results. The aim was to clarify the involvement of HPV among lesions observed. Most of cervix cell alterations were represented by ASCUS (43.2%). Out of 1836 β-globin positive samples, HPV genotypes were found in 1484 samples giving a HPV prevalence of 76.2%. Low Risk HPV genotypes were identified in 75.51% of all positive samples. 297 cases of multiple infections were recorded in the study and were mainly represented by co-infection with genotypes of low risk. This table presents characterization of women for molecular analysis. We selected 1947 women with positive uterine cell alteration according to VIA/VILI or Pap Test. Out of 4063 women fulfilled inclusion criteria. 1947 presented uterine cell alterations according to VIA/VILI or Pap test and were therefore included in this study. The prevalence of ASCUS (61.8%) (P value < 0.001), LSIL (43.2%) (P value < 0.001), and HSIL (59.4%)(P value < 0.001), were higher in women from Yaounde than in their counterparts from, Niete and Mokolo. In addition, ASCH lesions were more frequent in Mokolo (40.6%) as summarized in table below.

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Table 1: Cytology and PCR results

		Effective	Percentage	95% CI	P value
Cytology Diagnose					
	ASCUS	841	43.2	35.3-51.7	
	ASCH	653	33.5	26.1-40.4	
	LSIL	319	16.4	9.4-23.6	< 0.001
	HSIL	134	6.9	2.6-15.1	
β-globine Presence					
	β-globine+	1836	94.2	82.4-97.3	< 0.001
	β-globine-	111	5.8	3.2-13.6	
HPV Statut					
	HPV+	1484	76.2	70.1-82.5	
	HPV-	256	13.1	7.4-17.9	
	Unsatisfactory smears	207	10.6	4.6-18.3	< 0.001
HPV Genotypes according to classes					
	LR-HPV	1138	75.5	71.2 – 78.1	
	HR-HPV	346	23.4	17.3 – 29.5	< 0.005
Multiple Infections					
	LR-HPV + HR-HPV	91	31	25.1 – 36.8	NS
	LR-HPV	144	48.2	45.7 – 54.4	
	HR-HPV	62	20.6	14.7- 24.8	

Study sites	ASCUS N (%) (n=1357)	ASCH N (%) (n=1439)	LSIL N (%) (n=957)	HSIL N (%) (n=310)
NYETE	229 (16.8)	321 (22.3)	314 (32.8)	65 (20.9)
MOKOLO	288 (21.2)	586 (40.7)	231 (24.1)	63 (20.3)
YAOUNDE	840 (61.9)	532 (36.9)	412 (43)	182 (58.7)
P value	< 0.001			

NS: Non Significant
ANOVA: One way was the test used
LR-HPV: Low Risk Human Papilloma Virus
HR-HPV: High Risk Human Papilloma Virus
ASCUS: Atypical Squamous Cells Undetermined Significance
ASCH: Atypical Squamous Cell not exclude HSIL
LSIL: Low Grade Squamous Intraepithelial Lesion
HSIL: High Grade Squamous Intraepithelial Lesion

b) Frequency of High Risk genotypes (HR-HPV) identified

Table 2 below present different cervical lesions and HR-HPV genotypes identified. The prevalence of low risk genotypes was higher than of high risk ones in ASCUS, ASCH and LSIL lesions. Conversely, high risk genotypes were more frequently associated with HSIL lesions than Low risk genotypes (20%, versus 6.1%) as presented in table 2. However, the difference was not statistically significant (P value=0.0847). ASCUS (60%); was more representative among women with Genotype 16 no patient with LSIL had the HR 16 genotype. The HR 18 genotype was identified in among all women presenting lesions, with the most prevalence for HSIL (30%). HPV 35 was identified mostly among women with LSIL (80%). The final general analysis shows a high frequency of HR 45 genotypes among all the women selected; in fact, HPV 45 was more representative among women who had presented ASCUS (54.5%).

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Table 2: HR-HPV genotypes and precancerous cervical lesions

HR Genotypes		HPV 16 (n=54)	HPV 18 (n=100)	HPV 35 (n=52)	HPV 45 (n=105)	HPV 58 (n=0)	HPV 84 (n=35)
		N(%)	N(%)	N(%)	N(%)	N(%)	N(%)
Precancerous cervical Lesions	ASCUS	32 (59.3)	20 (20)	0(0)	55 (52.4)	0 (0)	11(31.4)
	ASCH	12 (22.2)	28 (28)	0(0)	10 (9.5)	0(0)	9 (25.7)
	LSIL	0(0)	24 (24)	42 (80.8)	23 (21.0)	0(0)	10 (28.6)
	HSIL	10 (18.5)	28 (28)	10 (19.2)	17 (17.1)	0(0)	5 (14.3)
	Total	54 (100)	100 (100)	52 (100)	105 (100)	0(0)	35 (100)
	P value			< 0.001			

ASCUS: Atypical Squamous Cells Undetermined Significance
ASCH: Atypical Squamous Cell not exclude HSIL
LSIL: Low Grade Squamous Intraepithelial Lesion
HSIL: High Grade Squamous Intraepithelial Lesion
HR: High Risk
HPV: Human Papilloma Virus

c) *Prevalence of Human papilloma virus infection High risk among Cameroonian women*

Figure 1 below presented different high risk virus identified during the study. HPV 45 and HPV 18 were the most represented with 30.3% and 28.9% respectively.

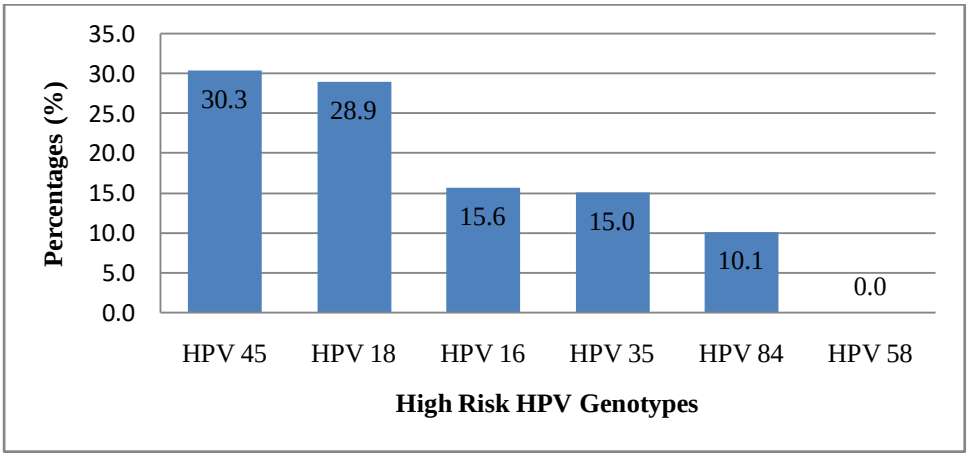

Figure 1: Prevalence of different HR-HPV genotypes targeted in the study population

d) *Frequency of Low Risk genotypes (HR-HPV) identified*

Table3 below presented cervical lesion according to HPV genotype. The distribution of all low risk HPV genotypes targeted in this was significantly unbalanced as presented in Table 5 (P-value<0.0001).

Table 3: LR-HPV genotypes and precancerous cervical lesions

LR Genotypes		HPV 6 (n=294)	HPV 11 (n=190)	HPV 61 (n=218)	HPV 62 (n=138)	HPV 70 (n=139)	HPV 81 (n=159)
		N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Precancerous cervical Lesions	ASCUS	110 (37.4)	71 (37.4)	90 (41.3)	37 (26.8)	80 (57.6)	28 (17.6)
	ASCH	91 (31)	25 (13.2)	50 (22.9)	26 (18.8)	52 (37.4)	69 (43.4)
	LSIL	83 (28.2)	52 (27.4)	78 (35.8)	75 (54.3)	0 (0)	53 (33.3)
	HSIL	10 (3.4)	42 (22.1)	0 (0)	0 (0)	7 (5)	9 (5.7)
	Total	294 (100)	190 (100)	218 (100)	138 (100)	139 (100)	159 (100)
	P value			< 0.001			

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e) Prevalence of Human papilloma virus infection Low risk among Cameroonian women

Figure 2 below present distribution of HPV. On the six low risk HPV genotypes targeted, HPV 6 and HPV 61 were the most represented with 25.8% and 19.2% respectively.

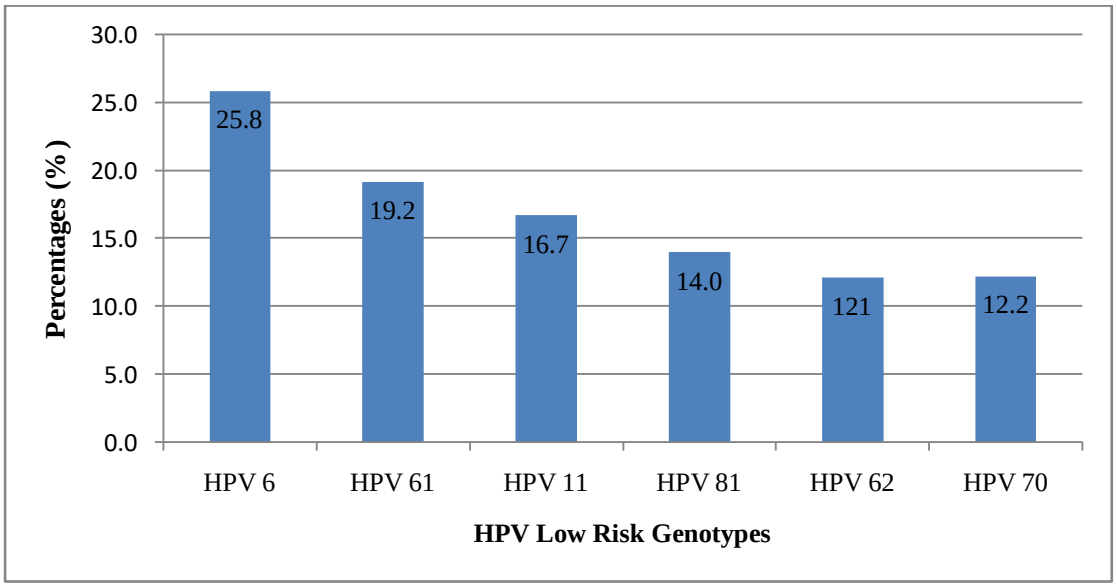

Figure 2: Frequency of distribution of LR-HPV genotypes targeted in the study population

f) Genotype and cervical lesions general association

This figure3 present association between HPV genotypes and precancerous lesions; according to this PCA (principal component analysis) above, many HPV infected are related to specific cervical cells lesions. Appearance of ASCUS is most correlated with HPV genotypes 84 (P value < 0.001). HSIL and LSIL could be respectively associated with HPV genotype 45 and genotype 16 significantly (P value < 0.001).

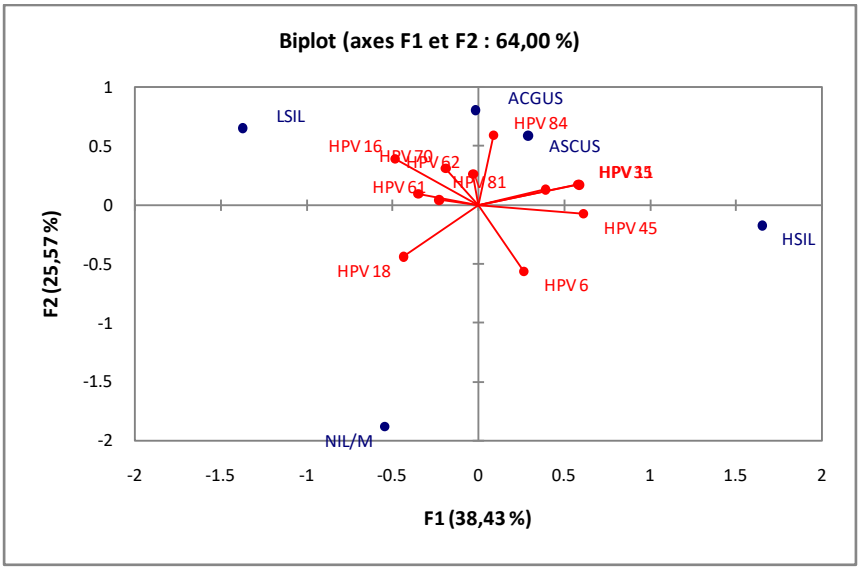

Figure 3: Principal component analysis (PCA) of HPV genotype and precancerous cervical lesions

IV. Discussion

The aim of this study was to characterize Human Papilloma Virus genotypes and their prevalence among Cameroonian women living with precancerous cervical lesions. For this purpose, additional analyses were carried out in samples of patients with lesions (ASCUS to HSIL). Out of 4063 patients with ASCUS, ASCH, LSIL and HSIL we selected, 1947samples were PCR positive. Various lesions were identified and were classified according to the Bethesda classification system. 76% of the samples analyzed were positive for at least one genotype of Human Papilloma Virus. However, remaining samples were negative after PCR. This finding is consistent with previous studies [6], [11]. Indeed, HPV presence on these samples presents his

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part of contribution during carcinogenesis. Multiples infections with HR and LR-HPV accounted for 31.03% of all mixed infections[13]. Low Risk HPV genotype 6 was the most prevalent in Cameroon, previous studies presented similar finding[14].HSIL precancerous lesions were found in 20% of women carrying High Risk HPV; almost identical to those found by Desruisseau and collaborators in 2009 [3]. Assessment of HPV infection according to lesions shows that, more women with cervical lesions were also infected by HPV subtypes; this situation can be explain by capacities of lesions to allow HPV penetration on the cervical cell. Results obtained in Iran showed almost the same results [15]. This frequency of lesions is not unique and china study has presented similar data[16]. The HR HPV 18 genotype was identified among women presenting LSIL and HSIL; many studies identified the HPV 18 as the most characterized on HSIL samples [15], this may be due to the dynamic of the virus to modify his polymorphism according to the environment. The final global analysis shows a high frequency of HR genotypes 45 (31.42%), followed directly by the genotype 18 (28.57%). HPV 45 and HPV 18 are two HPV genotypes which belong to HPV High Risk Group; their most prevalence could be attributed to interact with host cell domain and lead to degradation of PDZ-domain-containing proteins through its Carboxy-terminal motif [2].

Among women with low-risk genotypes, the highest frequency was observed among women with indeterminate lesions ASCUS and ASCH (63.9%) as in other studies [16] ; this observation present the capacity of the virus to destroy the cervical tissue and to induce dysplasia, as shown Muentes et al.,[18]. However, a study by Castle et al showed that the most prevalent HPV types in the vaginal samples were HPV71 (4.0%), HPV61 (3, 7%) and HPV58 (3.4%) [19]. No patient with genotype 11 had LSIL; this result presents genotype 11 not so implicated in the development of LSIL in this Country, may be because the genotype 11 is identify as low risk and less dangerous. The final general analysis shows a high frequency of LR 6 genotypes (25.4%), followed directly by genotype 61 (18.42%). Genotype 16 and other genotypes have a considerable frequency of occurrence in the study population. The highest frequency of HPV types found in this study were HPV45 and HPV18, HPV6 do not differ too much from those described in Africa[19], in Ethiopia[20], Morocco[7], Uganda [21], Algeria [8].However, the higher frequency of HPV6 found here is different from previous reports in other African countries that found the most widespread type of HPV, HPV58 in Botswana [22], HPV45 in Cameroon [3], VPH53 in Gabon[23], HPV35 in Burkina Faso [24], HPV2 in Kenya [25]. Multiple infections cases were identified. The HPV-LR 11 genotype was found in two cases of co-infection, as in many studies [26]. Other HPV-LR genotypes were also observed in co-infection cases such as HPV-LR 61 / VPH-HR 84 (11.11%); HPV-LR 62 / HPV-HR 58 (11.11%); HPV-LR 81 / HPV-HR (11.11%). Cuschieri and collabator demonstrated that the prevalence of multiple HPV infections is often high 43.3% and the most prevalent type of HPV multiple infection was only HR-HPV types, with 23.3% and a frequency of multiple infections LR-HPV of 0.8% [18].

V. Conclusion

The aim of this study was to characterize HPV genotypes circulating among Cameroonian women and to identify precancerous cervical lesions involved. Results presented that, Cameroon contain a big diversity of HPV. Global results concerning assessment of prevalence of Low Risk-HPV and High Risk, presented that Human Papilloma Viruses are present in Cameroon with various genotypes, it is important to take into consideration these genotypes during the implementation of prophylactic strategy.

Declarations

Ethics approval and consent to participate

The study protocol was written based on the Helsinki ethical principles for medical researches and approved by the National Ethics Committee for Human Health Research
(n° 2014/08/485/CE/CNERSH/SP).

Consent for publication

Informed consent was obtained from all individual participants included in the study

Availability of data and materials

The data will be available upon reasonable request to the corresponding author

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Conflict of interest statement
"The authors declare no potential conflicts of interest."

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Leprosy: A Stander-By Public Health Contender in the Hard to Reach Communities along the Eastern Zone of Tanzania

By Madoshi, PB, Karuhanga, TA, Kaale, SE, Jullu, BS & Rev. Fr. Mpinge, S

ST Francis University

Abstract- *Background:* Leprosy is one of the neglected and poverty-related diseases in low and middle-income countries, the disease is associated with socioeconomic factors, poor knowledge of the victims to report cases and poor access to health facilities with a definitive diagnosis of the associated lesions. In most cases, patients are reluctant to report the infection earlier because of the insidious nature of the infection. Thus the study was conducted to determine the prevalence of leprosy in eastern, Tanzania.

Material and methods: A retrospective study design was undertaken by analysing the patient's biodata at Nazareth leprosy centre located in Ifakara Tanzania. The analysis was to determine the association of the disease with age, sex, location, type of lesions presented, disease detection technique and the time for reporting to the centre. The Chi-square and t-test were used to determine the association of the factors with disease causation at the p-value of 0.05.

Keywords: *nazareth ifakara, leprosy, SFUCHAS, public health, kilombero.*

GJMR-F Classification: *NLMC Code: WC 335*

LEPROSYASTANDERBYPUBLICHEALTHCONTENDERINTHEHARDTOREACHCOMMUNITIESALONGTHEEASTERNZONEOFTANZANIA

Strictly as per the compliance and regulations of:

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Leprosy: A Stander-By Public Health Contender in the Hard to Reach Communities along the Eastern Zone of Tanzania

Madoshi, PB [α], Karuhanga, TA [σ], Kaale [ρ], SE, Jullu, BS [ω] & Rev. Fr. Mpinge, S [¥]

Abstract- Background: Leprosy is one of the neglected and poverty-related diseases in low and middle-income countries, the disease is associated with socioeconomic factors, poor knowledge of the victims to report cases and poor access to health facilities with a definitive diagnosis of the associated lesions. In most cases, patients are reluctant to report the infection earlier because of the insidious nature of the infection. Thus the study was conducted to determine the prevalence of leprosy in eastern, Tanzania.

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Results: 157 patient data at Nazareth leprosy centre were analysed. More males (68.2%) reported to the centre than females, where males presented with critical cases were (72.6%). Mlimba district had more people reporting to the centre (44.6%) compared to the other districts around. The diagnosis was mainly based on presented clinical signs (62.4%) without skin smear slit.

Conclusion: The findings of this study outline importance of early detection, contact tracing of the patients and their relatives and treatment of diagnosed cases, this will contribute significantly to the control of the disease in the low resource communities.

Keywords: nazareth ifakara, leprosy, SFUCHAS, public health, kilombero.

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I. Introduction

Leprosy is also known as Hansen's disease is a chronic bacterial disease caused by *Mycobacterium leprae,* Gram-negative bacilli. The disease in untreated cases affects skin, eyes and periphery nerve injury leading to the deformity of extremities (Fischer M., 2017; Job et al., 2008). It remains a significant public health problem in the developing world, mainly due to the slow-growing nature of the aetiological agents leading to the long incubation period, thus the source of infection might be difficult to determine (Panda and Padhi, 2017; Turankar et al., 2019; Wheat et al., 2014). In addition, the golden standard for diagnosing leprosy is based on slit skin smear or nerve biopsy, followed by acid-fast staining (Fine 2006; Chandranesan et al., 2018) however in poor resource leprosy centres such diagnoses have been not effective instead the diagnosis require suspicious of the index based on clinical presentation.

Leprosy is among of the neglected diseases in developing countries, it mostly affects people in poor communities (Hotez & Kamath, 2009; WHO, 1998) The World Health Organisation has reported a 2.9 new case detection in 100,000 populations with a prevalence of 0.27 per 10,000 (WHO, 2015b, 2017). In Tanzania the national strategic plan for 2015 – 2020 (NTLP, 2015); reported that leprosy prevalence remained below 1 case per 10,000 populations since 2006; however, it was 0.46 cases per 10,000 populations in 2012. The report also states that leprosy is endemic in 19 administrative districts such that the prevalence rate is above the threshold of 1 case per 10,000 populations. Globally, the strategy to end leprosy was set commence in 2016 and end in 2020. This was aimed at reducing the level of infection so that there could be less than one per million as well as alleviating permanent disabilities in particular children (WHO, 2015a). This could not be made possible without early detection of cases through surveillance, detection and initiation of medication (Freitas et al., 2016; NTLP, 2015).

The National Tuberculosis and Leprosy Programme (NTLP) in Tanzania has established some centres which could the lepers as closer as possible either as diagnostic or drug refill centres and refresher training of health workers especially leprosy

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coordinators. Despite such efforts, there are cases reported; for example, there were 2005 new cases in 2013 with children being infected (URT, 2013). As for the Grade, 2 disabilities is concerned, the national strategic reports a high disability rate of 10 – 12% among newly diagnosed patients, besides the year 2015 was aimed at reducing the grade 2 disability by 8% (URT, 2013).

The efforts to the lender the world free of leprosy prevalence are enormous. However, the reduction in the transmission and incidence of the disease is awaited. The impediment to ending the disease could be due to the fact that disease prevalence is not a comprehensive measure to end a particular disease since it is influenced by the duration of treating the disease and case identification. For a long term disease, the alternative method to present the disease is by using the rate of new cases with grade 2 disability (Alberts et al., 2011; Freitas et al., 2016; Yadav et al., 2014). The study aimed at describing the trends of the main indicators of leprosy and the rate of new cases from medical records to present and leprosy centre emergence and transmission of leprosy in eastern Tanzania.

II. Material and Methods

a) Study area

This work utilised reliable clinical records stored at Nazareth leprosy Centre (NLC) in Morogoro, Tanzania. NLC is a non for profit centre under the Catholic Diocese of Ifakara located in Ifakara Township where it serves all leprosy cases along the Kilombero valley basin. It is one of the leprosy centres which serve leprosy patients not only along the Kilombero valley but as well to other regions in the east and central Tanzania. The centre provides a diagnosis of the newly reported patients, treatment of patients with deformities and drug refill.

b) Study design and population

A retrospective observational study was carried to determine the prevalence and risk factors of acquiring new infections of leprosy using medical records. The study population existed of all leprosy patients diagnosed and registered in the period of January 2018 to March 2021.

c) Case definition

A person is defined to be infected with *M. leprae* and being ill if he/she presents one or more features as well as who has not completed a full course of treatment following being positively diagnosed. The features are several but may include: Painless skin ulcers, skin lesions of hypopigmented macules, skin nodules and where possible detection of acid-fast-bacilli in the silt skin smears (WHO, 1998). Furthermore, the grading at the centre is done based on whether it is paucibacillary (PB) or Multibacillary (MB) or visible deformity on hands and or legs and visual impairment as described by WHO (2015) and (Brandsma & Van Brakel, 2003).

d) Mode of patient identification

The centre receives patients from those who voluntarily report, contact tracing or surveys and referred cases from other health facilities within the districts, region or outside the region. Most of those reported voluntarily to the centre had tried to get treatment in other health facilities but their efforts were in vain.

e) Data analysis

The patient biodata which was stored in hard copies were entered into Microsoft Excel and SPSS version 20 was used to analyse the data. The information were presented as descriptive statistics to determine the frequencies and distribution of different factors such as age, year, location, time, prior health centres attended, relapsing or non-relapsing and gender. An independent t-test was used to infer the statistical significance.

III. Results and Discussion

The study analysed patient biodata at Nazareth leprosy centre located in Ifakara, Morogoro, Tanzania. The medical records were assessed where information such as the location of the patient, age, disease history, diagnostic measures, and number of clinics attended before and treatment regime was undertaken. The results in Table 1 show that most of the patients were above 35 years 107 (68.2%) and males were mostly affected 114 (72.6%), they were mostly coming from Mlimba district 70 (44.6%), have primary education 129 (82.2%) and they are farmers 133 (84.7%)

Table 2 shows information related to the disease; the results reveal that there were 68 (43.3%) in 2020, in most cases the diagnosis is done based on clinical signs presented by the patients 98 (62.4%) such that no biopsy was collected. The patients also attend several clinics before the definitive diagnosis is reached 67 (42.7%), most of the patients are diagnosed after more than one year 62 (39.5%) and most of them fail to attend clinics for various reasons 85 (54.1%).

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Table 2: Disease information of the patients attending at NLC

Patient Factor	Category	Distribution (n, %)
Year of data recording	Jan - Dec, 2018	16 (10.2)
	Jan - Dec, 2019	64 (40.8)
	Jan - Dec, 2020	68 (43.3)
	March, 2021	09 (5.7)
Diagnostic technique used	Clinical signs	98 (62.4)
	Silt skin smear microscopy	34 (21.7)
	Both clinical signs & microscopy	25 (15.9)
Laboratory results after diagnosis	Paucibacillary	25 (15.9)
	Multibacillary	33 (21.0)
	No biopsy collected	99 (63.1)
Number of Clinic priory attended	Only one clinic	45 (28.7)
	Only two clinics	45 (28.7)
	Several clinics attended	67 (42.7)
Time taken for a definitive leprosy diagnosis	Within 6 months	55 (35.0)
	Within a year	40 (25.5)
	More than 1 year	62 (39.5)
Frequency of attending clinic	Only once	85 (54.1)
	Regularly	72 (45.9)
Number of deformity or skin lesion	Multiple deformities	32 (20.4)
	Single deformity	41 (26.1)
	Senseless hyperpigmented skin lesion only	84 (53.5)

Table 3 shows results of the patient information when compared with respect to the type of deformities among patients. It was noted that males had more deformities than females, Mlimba district had more patients with multiple deformities 17 (11.0%) as compared to other places, patients under 15 years mostly reported the cases when the primary leprosy lesion appears (skin hyperpigmentation) while most of the deformities were reported more on patients with an age range of 36 years and above

Table 3: Types of deformities with respect to gender, location and age of the patients

Factor	Category	Type of deformity		
		Multiple types (n, %)	Single type (n, %)	Skin hyperpigmentation only (n, %)
Gender	Male	23 (15)	30 (19)	61 (39)
	Female	9 (6)	11 (7)	23 (15)
Location	Ifakara	0 (0.0)	0 (0.0)	1 (1.0)
	Mlimba	17 (11.0)	18 (11.0)	35 (22.0)
	Kisaki	9 (6.0)	12 (8.0)	20 (13.0)
	Kilosa	5 (3.0)	7 (4.0)	17 (11.0)
	Other places	1 (1.0)	4 (3.0)	11 (7.0)
Age	Below 15 years	0 (0.0)	0 (0.0)	4 (3.0)
	15 – 35 years	7 (4.0)	19 (12.0)	20 (13.0)
	36 years and more	25 (16.0)	22 (14.0)	60 (38.0)

Table 4 shows the indicators of acquiring *M. leprae* infection with respect to location; it was shown that the Mlimba district had a high proportion of indicators of leprosy. These included new cases (17.5),

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case detection rate (1.0/10,000 population), the proportion of female (0.3) and male (5.0) patients and the proportion of new cases presenting grade 2 lesions (7.0). Notably, the new cases and cases of patients presenting with grade 2 deformities were detected in all places.

Table 4: Indicators of leprosy of patients reporting or referred at NLC, Ifakara

Leprosy indicators of patients at NLC, Ifakara	Location				
	Ifakara	Mlimba	Kisaki	Kilosa	Other places
New cases detection (average cases in 4 years)	0.3	17.5	10.5	7.5	4.0
The New Case Detection Rate per 100,000 population	0.0	3.0	1.0	1.0	0.0
The proportion of children below 15 year old per year (n = 4)	30%	70%	0.0	0.0	0.0
The number of proportion Female patients per year in percentage (n = 43)	0.0	30%	40%	10%	10%
The number of proportion Male patients per year in percentage (n = 114)	0.0	60%	30%	20%	10%
The Multibacillary proportion of new cases in percentage (n = 33)	0.0	50%	30%	1.0%	1.0%
The proportion of new cases presenting patients with grade 2 disabilities (10%)	0.1	7.0	4.1	2.9	1.6

Leprosy in Tanzania is going down as reported by the national data, it has been pointed that in 2020 a total 1,208 new leprosy cases in the country with annual notification rate (case detection rate) of 2.6/100,000 (*http://.www.ntlp.go.tz/leprosy/leprosy-burden*). However, based on our data which were consecutively collected for three years from one of the leprosy centres in Tanzania. It can be generally stated that leprosy is has been dealt accordingly national wide; although the efforts made seem not eliminated the disease; the data presented in this study, it is either the disease is re-emerging or the pathogen has gained other pathogenic traits to cause infection while evading the prescribed drugs. In addition, the study has shown that there are new cases with grade 2 disabilities and the existence of hotspots for leprosy infection.

The occurrence of the hotspot for leprosy has been also reported by other researchers; Freitas et al., (2016) observed an increase in the incidence of leprosy in some local hotspots when with communities to diagnose leprosy in Brazil. (Aceng et al., 2019) analysed leprosy data in Uganda described that leprosy cases were clusters more in the Northern region. However, the authors commented that the endemicity of leprosy could result in establishing better health-seeking behaviour and case identification by training more health workers in highly endemic spots. (Joshua et al., 2008) worked on data collected in different health facilities in South India commented that leprosy in endemic areas is dealt with by spatially clustering the cases; this allows understanding the variation of the disease over space and time.

Our findings have also shown that leprosy is active even in children of less than 15 which is one of the good indicators of active leprosy transmission (Bandeira et al., 2019; Liu et al., 2018; Narang & Kumar, 2019). This finding has also been reported in Zambia whereby data of nearly 20 years (1991 – 2020) showed that there was active transmission among children; the scenario was also associated with late diagnosis as well as detection bias (Kapata et al., 2012). Msyamboza et al., (2012) carried a community camp-based study on the prevalence of leprosy in Malawi and 24.3% of the patients were children under the age of 15 years. A study by Bandeira et al., (2019) in Brazil reported 33.3% infection in 34 children below 15 years; the authors also quantified that those children had even Grade 2 deformities. Based on these studies it can be learnt that children might be the most vulnerable group to M. *leprae* infection due to the low incompetent immune system, interfamilial and intra-familial contacts (Narang & Kumar, 2019). The authors as well commented that the skills for diagnosing and managing leprosy are diminishing resulting in patients developing grade 2 deformities. Thus our findings envisage that for all efforts undertaken for leprosy elimination, new approaches are required under field condition to break the chain of transmission as well as prevention of new infections in children.

Our findings have shown that the number of males who reported to the health facilities for further diagnosis was high as compared to females. This scenario has also been found by other researchers: (Liu et al., 2018) in China assessed the difference of gender in leprosy detection, the authors concluded that more

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females were detected. Ramos et al., (2012) worked on leprosy gender differentiation and concluded that most of the patients recruited in their study females were relatively younger than male participants. Globally, leprosy distribution between males and females has a ratio of 2:1 meaning that there are more men with leprosy as compared to their female counterparts. The disease in the poor resource communities has been associated with (i) socio-economic; Nery et al., (2019) reported that individuals living in high poverty had a risk of leprosy incidence five-to-eight times greater than other individuals, (ii) pathogen's long incubation period (2 – 12) years hinders the effort of the World Health Organisation (WHO) to eliminate the disease and (iii) gaps on knowledge with respect to individual susceptibility to infection and disease development in newly affected patients (Rodrigues & Lockwood, 2011). However, there is no commonly identified socio-economic determinant that has been precisely described to cause leprosy transmission in such vulnerable and marginalised communities (Houweling et al., 2016).

In this study most of the patients who reported to the leprosy centre and started taking the medication were male. This could be speculated using different approaches; firstly male patients are more mobile than female patients who are tailored with house works and children bearing than men. Secondly, the disease affects more men in the respective areas due to their general behaviour in most African societies, such that males regularly intermingle with different people during their economic activities as compared to females, and thirdly the bacteria *M. leprae* might be gender-related, that is affecting more males than females. The results have also shown that most of the affected individuals who reported the clinical signs were peasant, this is probably conceding with the economic activities of such population as described by (Nery et al., 2019). Surprisingly more cases were from the Mlimba district; this could be hardly explained since the rationale for the spatial inequality on the distribution of the disease in the hyperendemic pockets within a country is untold (Ploemacher et al., 2020). Thus it is an area of interesting research to quantify the association of a disease occurrence with geographical factors associated.

Apparently, it is true that leprosy incidences are reported to be falling, however, the detection rate of the new case is dramatically low in most of the health facilities, as result most patients report to health facilities after noticing single or multiple disabilities. This could either be associated mainly with late reporting to respective leprosy centres, failure to monitor cases or the knowledge of health workers to diagnose leprosy is lacking. This situation has also been reported by Freitas et al., (2016) who were working on aggregated municipalities in Brazil. Aceng et al., (2019) established the knowledge and skills of health care providers even in highly endemic areas is limited; in such a situation the diagnosis is based only on multibacillary cases and observed disability among patients. Furthermore, the authors mentioned that the lack of such skills has led to a decrease in data collection coverage. The findings by Msyamboza et al. (2012) working on community camp-based in Malawi also supported the use of cohort analysis in patients with leprosy.

IV. Study Limitation

The study reproducibility might be limited with the fact that it used secondary data, which by considering leprosy and the living conditions of people in hard to reach communities it might lead to underreporting of the disease due to the insidious nature of the disease. Leprosy is associated with a long incubation period, the complexity of the transmission chain, asymptomatic state and that its diagnosis requires specific skills, training and experience of the one making the diagnosis (Limeira et al., 2013; Meima et al., 2004). On contrary, the presentation of patients with grade 2 disabilities could just be the significance of using secondary data as it is expected that underreporting can be seldom done as the signs are evident, the authors also recommend contact tracing of patient relatives.

V. Conclusion

Leprosy is now regarded by different communities as a disease of the past, despite the new cases being diagnosed in different locations. However the scenario is alarming especially in resource limited communities, thus the disease is re-emerging at a relatively high rate than anticipated. It can be concluded that the laxity to provide appropriate public health education and awareness creation shall worsen the situation in populations at risk. Therefore efforts are required by both the government and general public to work in tandem to provide public health, early diagnosis and appropriate treatment of the population at risk.

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Table 1: Demographic parameters of patients with leprosy

Patient Factor	Category	Distribution (n, %)
Age	Under15 years	04 (2.5)
	15-35 years	46 (29.3)
	Above36 years	107 (68.2)
Gender	Male	114 (72.6)
	Female	43 (27.4)
Location	Ifakara Township	01 (0.6)
	Mlimba district	70 (44.6)
	Kisaki	41 (26.1)
	Kilosa district	29 (18.5)
	Other places	16 (10.2)
Education level	Primary education	129 (82.2)
	Secondary education	25 (15.9)
	Trained or graduates	03 (1.9)
Occupation	Peasant	133 (84.7)
	Employed	20 (12.7)
	School age	04 (2.5)

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Assessment of Long Lasting Insecticide Treated Net among Women of Child Bearing Age in a Community in South Eastern Nigeria

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Abstract- Globally, there have been attempts towards improving the coverage of malaria preventive measures with the 2015 goal of the World Health Organization's (WHO's) Roll Back Malaria Partnership centered to reduce global malaria cases by 75% and to reduce malaria deaths to near zero through universal coverage by effective prevention and treatment interventions. Regrettably, malaria still constitutes a serious public health problem in Nigeria. The aim of this study was to assess long lasting insecticide treated net use among women of child bearing age in Nwangele LGA Imo State. A community based descriptive cross sectional study was used for the study. The study involved women of child bearing age aged 15-45yrs at Nwangele LGA as its Target Population. A Probability based multi stage sampling method was adopted for the study. A semi-structured questionnaire was used as the instrument of data collection for this study and Statistical Package for the Social Sciences (SPSS) was used in the analysis of the data gotten from the study. A total of 404 women participated in the study.

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Assessment of Long Lasting Insecticide Treated Net among Women of Child Bearing Age in a Community in South Eastern Nigeria

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Abstract- Globally, there have been attempts towards improving the coverage of malaria preventive measures with the 2015 goal of the World Health Organization's (WHO's) Roll Back Malaria Partnership centered to reduce global malaria cases by 75% and to reduce malaria deaths to near zero through universal coverage by effective prevention and treatment interventions. Regrettably, malaria still constitutes a serious public health problem in Nigeria. The aim of this study was to assess long lasting insecticide treated net use among women of child bearing age in Nwangele LGA Imo State. A community based descriptive cross sectional study was used for the study. The study involved women of child bearing age aged 15-45yrs at Nwangele LGA as its Target Population. A Probability based multi stage sampling method was adopted for the study. A semi-structured questionnaire was used as the instrument of data collection for this study and Statistical Package for the Social Sciences (SPSS) was used in the analysis of the data gotten from the study. A total of 404 women participated in the study. Results from the study revealed that majority 41.5% (169) of the women were aged between 26-30 years. The study showed that a majority of the respondents with 96.5% (390) are aware and have had heard about malaria prior to the study. The study posited that 74.0% (299) of the participants had knowledge of long lasting insecticide treated nets. Further results of the study showed that 74.0% (299) of the participants had knowledge of long lasting insecticide treated nets. 48.0% (194) agreed towards distribution of LLITNs in the community. Based on the Level of Utilization of Long Lasting Insecticide-Treated Nets among the women, the study revealed that 44.3% (179) of the women said yes when asked if they had ever slept under an LLITN. Considering the association demographic characteristics and utilization of LLITNs the study found that marital status (p=0.004), level of education (p=0.0001) and income level (p=0.006) were associated with uptake of bed nets among pregnant women. The study concluded that the coverage and distribution of long lasting insecticide treated nets in the community among women had low ownership and distribution forming part of the component integration strategy of Malaria prevention. The study recommended that the federal government should train and empower a skilled manpower in surveillance and frequent check up of distribution gaps in LLITNs in the rural communities through the help of agencies and concerned nongovernmental organizations.

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I. Introduction

According to a publication by center for Disease Control (2014), malaria infection is caused by a protozoan (Plasmodiae). However the report posited that malaria infection occurs when favorable environmental conditions of temperature, rainfall, and humidity are created for the female Anopheles mosquitoes, carrying the Plasmodium, to bite a susceptible host (CDC, 2014). Malaria infection is endemic in Nigeria, with a prevalence of 919 per 10,000 of population; it remains one of the leading causes of morbidity and mortality (Ganihu & Jimo, 2013; Oche *et al.*, 2011; Aribodor *et al.,* 2017) It accounts for seven out of ten outpatient visits in Nigerian hospitals as well as being responsible for about 20% and 30% of infant and under-5 mortality rate, respectively (Oche *et al.,* 2011).

Following the attempts towards improving the coverage of malaria preventive measures, the 2015 goal of the World Health Organization's (WHO's) Roll Back Malaria Partnership are to reduce global malaria cases by 75% and to reduce malaria deaths to near zero through universal coverage by effective prevention and treatment interventions (RBM, 2010). Among other preventive interventions, WHO recommends the use of insecticide treated nets (ITNs), particularly Long-Lasting Insecticide Nets, which have been shown to be cost-effective, to reduce malaria episodes among children under 5 years of age by approximately 50% and all-cause mortality by 17%. Universal coverage with ITNs is defined as use by > 80% of individuals in populations at risk (WHO, 2019; RBM, 2010).

Aribodor *et al.* (2017) in a report posited that the usage of long lasting insecticide treated nets is largely

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affected by distribution patterns and also the knowledge of people and their perception about it. Behavioral patterns of people-utilization of the LLITNs are dependent on their socio demographic characteristics on the consequence of nonuse (Aina and Ayeni, 2011; Mbanugo and Okorudo, 2015; Aribodor *et al.,* 2017). Researchers give varied indications on the coverage and use of the LLITNs in various parts of the World and peoples level of knowledge it (Runsewe-Abiodun *et al.,* 2012; Aluko & Oluwatosin, 2012; Iwu *et al.,* 2010: Aina and Ayeni, 2011: Ganihu & Jimo, 2013). Isah and Nwobodo (2009) reported that despite evidence that the use of LLITNs decreases malaria-related morbidity and mortality, the use of LLITNs in Africa remains relatively low. Estimates suggest that in 2005, only 3% of children under five years of age slept under LLITNs, while up to ten times as many are thought to sleep under any bed net (Baley & Deressa, 2008). This shows that the fact that ITNs are very effective in malaria prevention does not necessarily mean that people will use them after they have received those (Baley & Deressa, 2008). While the evidence based on the effectiveness of LLITNs in reducing malaria transmission has grown rapidly in recent years, utilization rates of LLITNs in most African countries have been very low (Chukwuocha *et al.,* 2010; Ganihu & Jimo, 2013). The renewed Abuja, Nigeria, target for roll back malaria (RBM), a control program for malaria, targeted 80.0% of children <5 years of age and pregnant women to use long lasting insecticide-treated mosquito nets (LLITN) between 2006 and 2010 (FMOH, 2015; (Deneye *et al.,* 2011).

The millennium development goal 6 has a target of halting and beginning to reverse the incidence of malaria in 2015 (Baley & Deressa, 2008). These control programs are aimed at reducing the morbidity and mortality, resulting from malaria infections in at-risk groups particularly at Households. The past decades have witnessed an increase in international funding for malaria control. This increased funding has led to an increase in accessing LLITNs in Sub-Saharan Africa (Deneye *et al.,* 2011). At the end of 2010, approximately 289 million LLITNs were delivered to the Several Households at Sub-Saharan African region; this is enough to take care of 76% of the 765 million persons at risk (Deneye*et al.,* 2011). Insecticide Treated Nets is currently one of the most cost-effective options for reducing malaria-related morbidity and mortality and has been reported to reduce malaria mortality by 17% in children <5 years of age (Runsewe-Abiodun *et al.,* 2012).

Regrettably, malaria still constitutes a serious public health problem in Nigeria (Aina and Ayeni, 2011; Mbanugo and Okorudo, 2015; Aribodor *et al.,* 2017). Malaria is endemic in the poorest countries in the world, causing 400 to 900 million clinical cases and up to 2.7 million deaths each year (Guyatt & Ochola, 2014). More than 90% of malaria deaths occur in Sub-Saharan Africa, resulting in an estimated 3,000 deaths each day. Almost all the deaths are among high-risk groups including women of childbearing age, women during pregnancy, non immune travelers, refugees and other displaced persons, and people of all ages living in Household areas of unstable malaria transmission (Mbanugo & Okorudo, 2015; Iwu *et al.,* 2010: Aina & Ayeni, 2011: Ganihu & Jimo, 2013). In highly endemic countries, malaria poses a serious danger to women of child bearing age, women in pregnancy and their unborn children (Mbanugo & Okorudo, 2015). Malaria in pregnancy causes maternal anaemia, miscarriage, and low birth weight. In endemic countries, it is the leading cause of maternal mortality and one of the primary causes of neonatal deaths (Mbanugo & Okorudo, 2015).

According to some reports in Nigeria, malaria is the leading cause of Maternal Mobility contributing 33% of deaths among women of child bearing age and 25% infant mortality (Oche *et al.,* 2011; Iwu *et al.,* 2010). These Problems resulting due to minimal preventive measures especially in poor sub Saharan African regions like Nigeria which include low utility of LLITNs and poor sanitary conditions at Household and residential areas remain an issue of concern and a problem. Despite these interventions, women of child bearing age have reported gaps in uptake noting that LLITNs are effective in the prevention of malaria, ITN coverage and utilization still remain low in Nigeria with few studies have documented household net coverage and utilization in Nigeria (Aina and Ayeni, 2011; Mbanugo and Okorudo, 2015; Aribodor *et al.,* 2017). Most of the published studies available were conducted in other malaria endemic countries in Sub-Saharan Africa and the few published studies in Nigeria were from the urban centers of other states. With women of childbearing age being at significant risk, it is imperative to examine this coverage of long lasting Insecticide treated nets in Nwangele. However, it is due to the magnitude of the problem that the researcher aims to find out Long Lasting Insecticide Treated Nets use among women of child bearing age in Nwangele LGA, Imo state South Eastern Nigeria.

II. Methods

a) Design

A community based descriptive cross sectional study was used for the study to determine Long Lasting Insecticide Treated Nets among women of child bearing age in Nwangele LGA.

b) Study Setting

Nwangele is a Local Government Area of Imo State, Nigeria. Its headquarters are in the town of Amaigbo. Nwangele Local Government is administered under the terms of the Constitution of the Federal Republic of Nigeria. Imo State it is located at the central part of Amaigbo and it is made up of 11 villages

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comprising of Amaigbo Community, Abba Community, Dimnanume community, Isu Ancient Kingdom Community, Umuozu Community, Isiala Umuozu Community, Umunakara Community, Umudurunna Community, Abajah Community, Ogwuaga/Ekitiafor Community and Umunna Community. The urban towns of Nwangele L.G.A. are Abajah, Isu, Amaigbo, Umuozu and Abah, the rest are more of rural towns. Going by the 2006 census, the population of Nwangele L.G.A. was 127,691 people divided as Male 65022, Female 62669 (National Population Commission, 2006). Nwangele Local Government Area Constitutes mainly of Igbo people situated in her Residential territories as well as other minor ethnicities. It has an area of 295 km^2 and a population of 99,265 at the 2006 census The Coordinates of Nwangele Local Government Area is given as 5.4166° N, 6.9853 °E.

c) Study Population

This study on Long Lasting Insecticide Treated Nets among women of child bearing age in Nwangele LGA involved women of child bearing age aged 15-45yrs at Nwangele LGA as its Target Population

d) Inclusion criteria

The study included the following

i. Women of child bearing age at Nwangele LGA who gave in their consent for the study.
ii. Women of child bearing age at Nwangele LGA who are adults aged 18-45years present as at the time of data collection.

e) Exclusion criteria

The study excluded the following;

i. Women of child bearing age who refused to give in their consent for the study
ii. Women of child bearing age who were lunatic, sick or disabled during the time of data collection.

f) Sampling

i. *Sample size Calculation*

The sample size was determined using the Yamene formula (1967) for sample size determination.

$$n = \frac{N}{1+Ne^2}$$

Where:
n is the desired sample size
N is the population size (12,389) = population of women of childbearing age at Nwangele LGA (NPC, 2010).
e is margin of error (0.05)
Therefore,
n = 392.30362210

Furthermore, to adjust for a 10% rate of non response and invalid response (i.e 90% expected response rate =0.9).

n= n/expected response rate
n =392/0.90= 435.5
n= 436

ii. *Sampling Methods*

A Probability based multi stage sampling method was adopted for the study on the coverage of Long Lasting Insecticide Treated Nets among women of child bearing age in Nwangele LGA.

First stage-*Selection of Communities:* A total of Three (3) Out of the communities in Nwangele LGA was selected by the researcher using simple random sampling via balloting to give every community an equal chance of selection. Second stage- *Selection of villages:* Three (2) villages each out of the total number of villages in the selected community was selected via simple random sampling using balloting giving every village in the selected community an equal chance of being selected. Third stage- *Selection of Streets:* A total of Five (5) streets each in the selected Six (6) villages were selected via simple random sampling (balloting) to give every street an equal chance of being selected. Fourth stage: *Selection of households:* A systematic probability sampling method was used to select each household in the selected streets giving each household an equal chance of selection. Fifth stage: *Selection of Respondents:* the researcher selected women of child bearing age in each household or any one present at the time of study. Selection of respondents was done via simple random sampling.

g) Instrument for data collection

A semi-structured questionnaire was used as the instrument of data collection for this study on the coverage of Long Lasting Insecticide Treated Nets among women of child bearing age in Nwangele LGA.

h) Validity of the instrument

The validity of the instrument of data collection by the researcher took the following shape; the questionnaire as the instrument of data collection was developed by researcher and submitted to the research supervisor for Face validity and proper scrutiny as well as two experts from department of public health for consensus validity in order to ensure that the questionnaire meets the objectives of study before reliability testing.

i) Reliability of Instrument

The Reliability of the instrument of data collection was determined using test retest method. Copies of the questionnaire were given to some respondents outside the area of study by the researcher. This area shared similar characteristics with Nwangele LGA that was used for this study. Chrombach alpha test was used to test for the reliability of the questionnaire to determine the consistency of the results with a reliability coefficient of 0.8 obtained.

j) Method of Data Collection

Data was obtained using an interviewer based semi structured questionnaire. This will be done with the aid of Two (2) field assistants who will be Hired and

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trained to aid the researcher in the data collection process.

k) Method of Data Analysis

The Statistical Package for the Social Sciences (SPSS) was used in the analysis of the data gotten from the study. Results will be expressed in percentages, frequencies, tables and charts (Descriptive Statistics). Chi square was used to test the hypothesis statement of the study (p=0.05).

l) Ethical Consideration

A letter of introduction and ethical clearance was obtained from the School of Postgraduate studies Ethical clearance committee in Federal University of Technology Owerri (FUTO) before the research was conducted. The purpose of the research was explained to each respondent and verbal informed consent obtained from them before inclusion into the study. Also, anonymity of the respondents was assured and ensured. The confidentiality of the information they gave was also maintained.

III. Results

A total of four hundred and thirty six (436) copies of questionnaires were distributed for the study. They were properly filled and crosschecked for correctness, and 404 questionnaires were retrieved and were used for the purpose of the analysis.

a) Socio Demographic Factors of the Respondents

From the table 1 below, 41.5% (169) of the respondents were aged between 26-30 years, 20.5% (83) between ages 15-20, 9.9% (40) were people in their early 20's (21-25), 14.3% (58) were between 31-40 years of age and 13.3% (54) were adults within 41-45 year age bracket. On ethnicity, 38.1% (154) opted for ethnic groups not listed but label 'others', 93.7% (379) were of the Igbo ethnic group, 1.9% (8) Yoruba, and 0.4% (2) of the respondents were Hausa/Fulani. On educational backgrounds, 30.9% (125) of the respondents had Informal education, 12.8% (52) had attained the Tertiary level of Education, and 22.2% (90) had primary education and 37.1% (150) of the respondents with secondary level of education. 50.7% (205) of the respondents did occupations not listed but label 'others', 17.3% (70) were civil servants, 18.8% (76) of the respondents housewives while just 13.1% (53) were self employed. On the marital status of the respondents, 26.2% (106) were widowed, 24.7% (100) were single, 24.5% (99) married while 12.6% (51) of the respondents were separated. 11.8% (48) opted to choose 'others'. 27.2% (110) of the respondents had an income level between 1-10,000, 21.7% (88) had an income level between 31,000-50,000, 20.2% (82) earned above 100,000, 17.0% (69) had an income level between 11,000-30,000, and the least percentage 13.6% (55) earned an income between 51,000-100,000.

Table 1: Socio Demographic Factors of the Respondents

Characteristics	Frequency (n=404)	Percentage (%)
Age		
15-20	83	20.5%
21-25	40	9.9%
26-30	169	41.5%
31-40	58	14.3%
41-45	54	13.3%
Total	**404**	**100**
Ethnicity		
Igbo	379	93.8%
Hausa/ Fulani	2	0.4%
Yoruba	8	1.9%
Others	15	3.7%
Total	**404**	**100**
Educational level		
Informal education	52	12.8%
Primary	90	22.2%
Secondary	150	37.1%
Tertiary	112	27.7%
Total	**404**	**100**
Occupation		
Self employed	53	13.1%
House wife	76	18.8%
Civil servant	70	17.3%

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Others	205	50.7%
Total	**404**	**100**
Parity		
None	92	22.7%
1-2	169	41.8%
3-5	40	9.9%
Above 5	103	25.4%
Total	**404**	**100**
Marital status		
Married	99	24.5%
Single	100	24.7%
Separated	51	12.6%
Widowed	106	26.2%
Others	48	11.8%
Total	**404**	**100**
Income Level		
1-10,000	110	27.2%
11,000-30,000	69	17.0%
31,000-50,000	88	21.7%
51,000-100,000	55	13.6%
Above 100,000	82	20.2%
Total	**404**	**100**

b) Level of Knowledge of Long Lasting Insecticide-Treated Nets

Table 2 considering the level of knowledge of long lasting insecticide treated nets, a majority of the respondents with 96.5% (390) said "Yes" when they were asked if they had heard about malaria at any time prior to the questionnaires, while a small 3.4% (14) denied. When asked if they had suffered from malaria, a majority if the respondents also with 95.2% (385) replied "Yes" while just 4.7% (19) said "No". 61.6% (249) of the respondents believe mosquito bites causes malaria, 26.2% (106) said malaria is caused by dirt/stagnant water, 9.6% (39) chose plasmodium organisms and 4.7% (19) said "germs". Upon question on how malaria is transmitted, 44.0% (178) opted to choose 'Bites of any Mosquito', 19.0% (77) said "Bites of insect which has bitten a malaria Patient", and 36.8% (149) opted for 'Stagnant water and unclean environment'. 74.0% (299) of the participants replied "Yes" when asked if they had heard about LLITNs, while 25.9% (105) said "No". 24.2% (98) had heard about LLTINs from Health centers, 15.3% (62) from the Media, 17.3% (70) from publications/journals, 18.5% (75) chose options not listed but label 'others', 13.6% (55) heard about LLTINs from school, while 10.8% (44) from Family/Friends. Based on LLITNs is Key in Prevention of Malaria due to its Durability, 75.2% (304) said "Yes", while 24.7% (100) said "No". On if LLITNs is effective in the Prevention of Malaria when it is air dried frequently 73.7% (298) replied "Yes" and 26.2% (106) of the respondents said "No".

Table 2: Level of Knowledge of Long Lasting Insecticide-Treated Nets

Variables	Frequency (n=404)	Percentage (%)
Heard about Malaria		
Yes	390	96.5%
No	14	3.4%
Total	**404**	**100**
Have you Suffered from Malaria before		
Yes	385	95.2%
No	19	4.7%
Total	**404**	**100**
What causes malaria		
Germs	19	4.7%
Dirts/Stagnant Water	106	26.2%
Mosquito Bites	249	61.6%
Plasmodium Organisms	39	9.6%
Total	**404**	**100**
How is malaria Transmitted		
Bites of any Mosquito	178	44.0%
Bites of insect which has bitten a malaria Patient	77	19.0%
Stagnant water and unclean environment	149	36.8%
Total	**404**	**100**

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Have your Heard about LLITN		
Yes	299	74.0%
No	105	25.9%
Total	**404**	**100**
Source of Information		
Health center	98	24.2%
Media	62	15.3%
Family/Friends	44	10.8%
Publications/Journals	70	17.3%
School	55	13.6%
Others	75	18.5%
Total	**404**	**100**
LLITNs is Key in Prevention of Malaria due to its Durability		
Yes	304	75.2%
No	100	24.7%
Total	**404**	**100**
LLITNs is effective in the Prevention of Malaria when it is air dried frequently		
Yes	298	73.7%
No	106	26.2%
Total	**404**	**100**

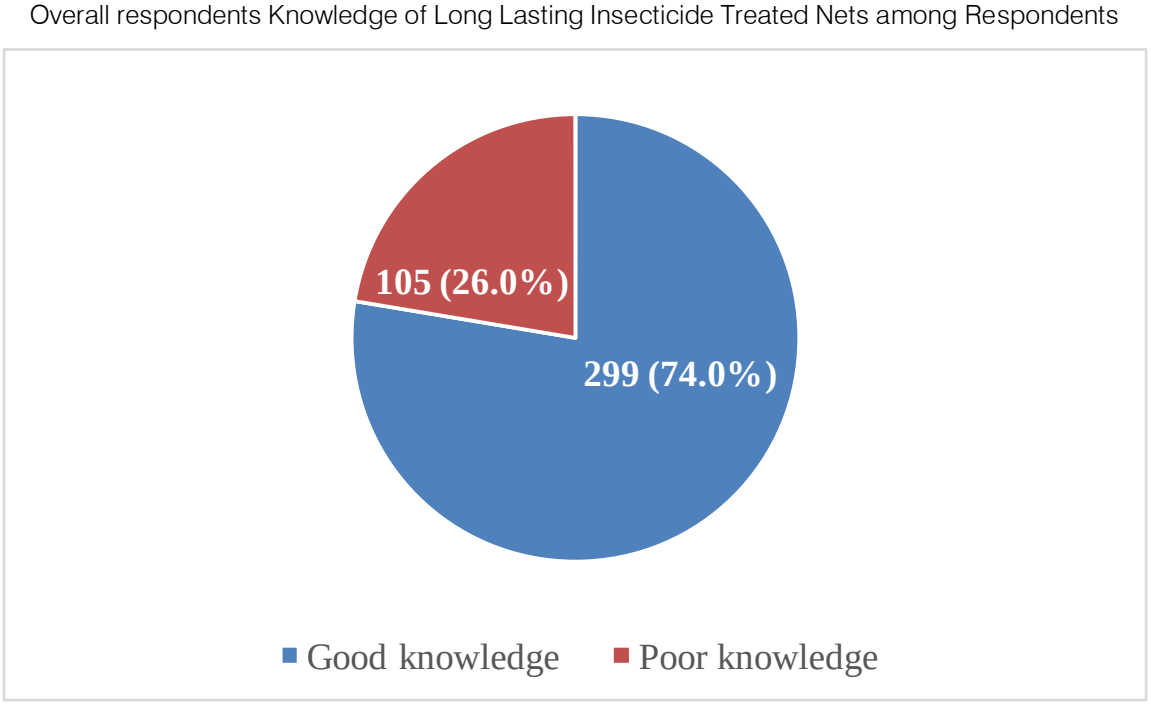

Figure 3: Overall respondents Knowledge of Long Lasting Insecticide Treated Nets among Respondents

Above figure 3 shows that 74.0% (299) of the respondents had good knowledge of long lasting insecticide Treated Nets among respondents while 109 (26.0%) had poor knowledge.

c) Distribution and Ownership of Long Lasting Insecticide- Treated Nets among Respondents

The table 3 below revealed the choices of respondents relative to Distribution and Ownership of Long Lasting Insecticide- Treated Nets. When asked if Insecticide Treated Nets had been distributed in their environs, 48.0% (194) replied "Yes" while 51.9% (210) said "No". When the respondents were asked if they had any Insecticide Treated Net, 71.2% (288) affirmed, while 28.7% (116) said "No". The respondents were asked if they owned a Long Lasting Insecticide Treated Nets, 45.0% of the respondents (182) said Yes, while 54.9% (222) denied. On the question 'How did you get it?', 38.3% (155) chose Health Centers, 33.1% (134) said "Market", 7.1% (29) said "Friends", 15.0% (61) of the respondents replied "School" while some respondents 6.1% (25) chose options not listed but label 'Others'. When asked How Many ITNs their household Owned, 41.3% (167) of the respondents said "None", 17.8% (72) replied "1", 29.9% (121) said between 2-4, 10.8% (44) of the respondents said "Above 4".

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Table 3: Distribution and Ownership of Long Lasting Insecticide- Treated Nets among Respondents

Variables	Frequency (n=404)	Percentage (%)
Insecticide Treated Nets distribution in your Area		
Yes	194	48.0%
No	210	51.9%
Total	**404**	**100**
Have any Insecticide Treated Net		
Yes	288	71.2%
No	116	28.7%
Total	**404**	**100**
Own Long Lasting Insecticide Treated Net		
Yes	182	45.0%
No	222	54.9%
Total	**404**	**100**
How did you get it		
Health Center	155	38.3%
Market	134	33.1%
Friend	29	7.1%
School	61	15.0%
Others	25	6.1%
Total	**404**	**100**
How Many ITNs do your household Own		
None	167	41.3%
1	72	17.8%
2-4	121	29.9%
Above 4	44	10.8%
Total	**404**	**100**

d) Level of Utilization of Long Lasting Insecticide-Treated Nets

Table 4 shows the Level of Utilization of Long Lasting Insecticide-Treated Nets among respondents. When asked if they had ever slept under an LLITN, 44.3% (179) said "Yes", while 55.6% (225) replied "No". The respondents were asked if they slept under an LLITN the previous night, 66.0% (267) confirmed, while 33.9% (137) denied. Respondents that denied were in turn asked when last they slept under an LLITN, 42.3% (58) told less than 7days ago, 33.5% (46) said between 8-29days, 24.0% (33) more than 30 days. When asked if their children/family members sleep under LLITN, 66.0% (267) confirmed "Yes", while 33.9% (137) said "No". The respondents who replied "No" were then asked if their children/family members slept under an LLITN the previous night, 62.8% (254) confirmed, while 37.1% (150) denied. Respondents that denied were in turn asked when last they slept under an LLITN, 32.0% (48) told less than 7days ago, 21.3% (32) said between 8-29days, 46.6% (70) more than 30 days.

Table 4: Level of Utilization of Long Lasting Insecticide-Treated Nets

Variable	Frequency	Percentage
Have you Ever Slept under an LLITN?		
Yes	179	44.3%
No	225	55.6%
Total	**404**	**100**
Did you sleep under an LLITN Last Night?		
Yes	267	66.0%
No	137	33.9%
Total	**404**	**100**
If No, when was the last time you slept under an LLITN?		
<7days ago	58	42.3%
8-29days	46	33.5%
> 30 days	33	24.0%
Total	**137**	**100**
Do your children/family Members sleep under LLITN		
Yes	267	66.0%

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No	137	33.9%
Total	**404**	**100**
Did they sleep under an LLITN last Night		
Yes	254	62.8%
No	150	37.1%
Total	**404**	**100**
If No, when was the last time they slept under an LLITN		
<7days ago	48	32.0%
8-29days	32	21.3%
> 30 days	70	46.6%
Total	**150**	**100**

e) *Factors Influencing the Coverage of Long Lasting Insecticide-Treated Nets*

Shown in table 5 below are the Factors Influencing the Coverage of Long Lasting Insecticide-Treated Nets among respondents. When asked if Nets Inflicts Rashes, 74.0% (299) of the respondents confirmed, while 25.9% (105) said "No". Considering the Distance to Facility, 62.8% (254) of the respondents said it was a factor, while 37.1% (150) did not consider distance to facility being a factor influencing coverage of LLITNs. When asked about Cultural Acceptance, 44.3% (179) said "Yes" while 55.6% (225) denied. The respondents were questioned concerning Family Factors; Study shows 71.2% (288) replied "Yes" while 28.7% (116) said "No". On whether Religious Acceptance influenced the use of LLITNs, 4.7% (19) affirmed "Yes" while majority of the respondents with 95.2% (385) said "No". 59.1% (239) denied Difficulty to install a LLITN was a factor influencing the use of the Nets, while 40.8% (165) confirmed. When asked if the respondents had door nets, 64.3% (260) said "Yes", while 35.6% (144) did not have door nets. On Information during Distribution being a factor influencing use of LLITNs was met with 44.0% (178) confirming among the respondents, while 55.9% (226) replied "No".

Table 5: Factors Influencing the Coverage of Long Lasting Insecticide-Treated Nets

Variable	Frequency (n=404)	Percentage (%)
Nets Inflicts Rashes		
Yes	299	74.0%
No	105	25.9%
Total	**404**	**100**
Distance to Facility		
Yes	254	62.8%
No	150	37.1%
Total	**404**	**100**
Cultural Acceptance		
Yes	179	44.3%
No	225	55.6%
Total	**404**	**100**
Family Factors		
Yes	288	71.2%
No	116	28.7%
Total	**404**	**100**
Religious Acceptance/Factors		
Yes	19	4.7%
No	385	95.2%
Total	**404**	**100**
Religious Acceptance		
Yes	194	48.0%
No	210	51.9%
Total	**404**	**100**
Difficulty to Hang		
Yes	165	40.8%
No	239	59.1%
Total	**404**	**100**
Have Door Nets		
Yes	260	64.3%
No	120	35.6%
Total	**404**	**100**

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Information during Distribution		
Yes	178	44.0%
No	226	55.9%
Total	**404**	**100**

f) Relationship between Socio-demographic characteristics and level of utilization of long lasting Insecticide Treated Nets

Based on the Relationship between socio-demographic characteristics and level of utilization of long lasting insecticide treated nets, the table below shows that age not significantly associated with level of utilization insecticide treated nets (P = 0.5301). Furthermore, the table 4.6 shows that religion is not significantly associated with utilization of LLITNs (P = 0.115). Also, ethnicity doesn't show significant association with utilization of LLITNs (P = 0.074). Marital status is significantly associated with utilization of LLITNs (P = 0.0001). Moving further, the table reveals that parity is significantly associated with the level of utilization of LLITNs (P = 0.0001). Also, level of education shows significant association with level of utilization of LLITNs (P = 0.0001). Occupation is not significantly associated with level of utilization of LLITNs (P = 0.942). The level of income of the respondents shows significant association with level of utilization of LLITNs (P = 0.006) (Table 6 below).

Table 6: Association between Socio-demographic characteristics and level of utilization of long lasting Insecticide Treated Nets

Characteristics	X^2	D.F	P value	Decision
Age	106.411	36	0.5301	NS
Religion	33.340	318	0.115	NS
Ethnicity	53.008	36	0.074	NS
Marital status	106.124	127	0.0001	S
Parity	81.645	36	0.0001	S
Education level	153.283	36	0.0001	S
Occupation	31.133	45	0.942	N.S
Level of income	71.977	45	0.006	S

IV. Discussion

Based on the finding of this study on Long Lasting Insecticide Treated Nets among women of child bearing age in Nwangele LGA, considering the socio demographic characteristics, it was revealed that majority 41.5% (169) of the women were aged between 26-30 years. This finding goes in consistent with a study by Odoko *et al.,* (2012), that women of child bearing age have a mean age of 32.4yrs. The study revealed that majority 93.8% (379) of the respondents were Igbo region. This could be due to the fact that the study was conducted in Nwangele LGA which is the southeastern part of Nigeria dominated by people of Igbo origin. The findings of the study revealed that 27.2% (110) of the respondents had an income level between 1-10,000 naira. A study by Kenneth and Amefume (2013) posited a significant improvement in income level among women in rural areas. However this goes in contrast with the study with women of child bearing age mostly involved in petty trading.

Considering the level of knowledge of long lasting insecticide treated nets, the study showed that a majority of the respondents with 96.5% (390) are aware and have had heard about malaria prior to the study in consistence with a similar study conducted among groups of women of childbearing age (WOCBA) in Malawi by Owen *et al* (2018) on the awareness of Malaria among pregnant women. Information on Malaria is now widely open with several source of information existing. The study posited that 74.0% (299) of the participants had knowledge of long lasting insecticide treated nets. Studies by Kyi *et al.* (2020) and Adebayo *et al* (2014) showed that respondents in an area had 69.6%, 81.5% respectively knowledge of long lasting insecticide treated nets which corroborates with the finding of this study. Women of child bearing age at Nwangele are more likely to get information on the utilization and adequate knowledge following community meetings, hospital visits and at educational institutions. The study further revealed that majority 24.2% (98) had heard about LLTINs from Health centers. This goes against a finding by Atenchong *et al* (2014) that revealed that majority of women had good knowledge of LLITNs and ITNs from a community follow up program. However from this study it implies that health workers proffer information to women on antenatal and other related periodic health visit by women of child bearing age in the community.

From the study it was revealed that the women when asked if Insecticide Treated Nets had been distributed in their environs, 48.0% (194) agreed. However this shows a poor reach of LLITNs in the community. The findings of this study on distribution of LLITNs reveal that women of child bearing age might experience shortfall of these LLITNs. A study by Kenneth and Amefume (2013) demonstrated that distribution of LLITNs was hugely affected in areas where concerned organizations seek for coverage. From the study 45.0% of the respondents (182) owned long lasting Insecticide treated nets in corroboration with a previous study by Owen *et al* (2018) on converge and ownership of LLITNs

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by women in rural areas of Nigeria. Decreased ownership has been stated by the World Health Organization in a recent publication (WHO, 2021). These could be due to a lot of factors which this study sought further to find 38.3% (155) of the women of child bearing age opined that they got LLITNs from Health Centers and 33.1% (134) said Market. A finding by Nankinga *et al* (2012) posited that majority of resident's uptake ITNs from the Markets which goes against the finding of this study among women of childbearing age.

Based on the Level of Utilization of Long Lasting Insecticide-Treated Nets among the women, the study revealed that 44.3% (179) of the women said yes when asked if they had ever slept under an LLITN which goes against the finding by Adebayo *et al* (2014) on utilization of insecticide treated nets. Several factors could be responsible for the low uptake of insecticide treated nets among the women which was investigated by the study. According to Bennett *et al*. (2012) usage of LLITNs can be harnessed by several factors which could include, itches, reactions etc.

Furthermore, based on the factors influencing the Coverage of Long Lasting Insecticide-Treated Nets among respondents, when asked if Nets Inflicts Rashes, 74.0% (299) of the respondents opined it does. This finding goes in consistent with previous studies on the factors influencing uptake of LLITNs among women (Iwu *et al.,* 2010: Aina & Ayeni, 2011: Ganihu & Jimo, 2013). However in this study, participants posited that Considering the Distance to Facility, 62.8% (254) of the respondents said it was a factor towards their utilization. This implies that the health center is situated far away from them. When asked about Cultural Acceptance, 55.6% (225) denied it could influence their utilization and the coverage of LLITNS in the area. This goes against a study conducted at rural Dars es Salaam that showed majority of respondents agreeing cultural acceptance as a modifier to Malaria preventive behavior. The study revealed also that information during Distribution being a factor influencing use of LLITNs was met with 44.0% (178) confirming among the women of child bearing age (Charles *et al.,* 2019). A publication by Kyi *et al.* (2020) revealed that source of information on malaria preventive approaches was imperative in determining its uptake. The finding of this study shows that for women of child bearing age, information on utilization is essential for them to utilize LLITNs.

Based on the Relationship between socio-demographic characteristics and level of utilization of long lasting insecticide treated nets, the study revealed that marital status is significantly associated with utilization of LLITNs (P = 0.0001). This implies that husbands acceptance of the utilization of LLITNs is a motivating factor. This goes in line with a study by Atenchong *et al* (2014) which found marital status to be associated with uptake of bed nets among pregnant women (P=0.004). Moving further, the study also demonstrated that parity is significantly associated with the level of utilization of LLITNs (P = 0.0001). This could be due to the fact that increasing number of children can lead to uptake as well as less number of children. This goes in contrast with a report published by Nankinga *et al* (2012) on Parity and Usage of Nets. Also, from the study among women of child bearing age in Nwangele, it was posited that level of education shows significant association with level of utilization of LLITNs (P = 0.0001). Women of child of bearing age with educational level and information on ITNs would likely utilize LLITNs. The study revealed that the level of income of the women shows significant association with level of utilization of LLITNs (P = 0.006). A study by Owen *et al* (2018) opined that women with higher income status could afford malaria preventive. This implies that from the findings of this study among women of child bearing age at Nwangele it is more likely for them to purchase LLITNs if they have the money.

V. Conclusion

However, from the study, the coverage and distribution of long lasting insecticide treated nets in Nwangele LGA forms part of the component of the 2011 RBM integration which is effective in rapidly increasing household possession and use of bed nets, achieving national bed net coverage goals set by National Health Development Plan (NHDP) 2012-2015. Low Ownership of LLITNs was reported in the study and malaria is a very serious public health problem; prompt treatment alone cannot guarantee the achievement of the goal. All strategies must be strengthened and employed in fight against malaria, if the desired goal is to be achieved. Findings from this study showed that majority of the residents had a considerable good knowledge of the use of insecticide treated nets but low ownership.

VI. Recommendations

The recommendations for this study include the following;

1. Health education on the effective use of long lasting insecticide treated nets among residents and practices to improve ownership and function.
2. Health facilities at Nwangele LGA should liaise with relevant stakeholders and authorities to improvise the distribution of safe insecticide treated nets to residents and patients affected.
3. The federal government should train and empower a skilled manpower in surveillance and frequent check up of distribution gaps in LLITNs in the rural communities through the help of agencies and concerned nongovernmental organizations.
4. Public Health Officers and Environmental health personnel's should develop policies that would mandate the government and legislative bodies to

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enact them to help protect the wider community at large.

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Questionnaire on the Evaluation of the Coverage of Long Lasting Insecticide Treated Nets Among Women of Child Bearing Age in Nwangele Lga, Imo State, South Eastern Nigeria

SECTION A: SOCIO DEMOGRAPHIC CHARACTERISTICS

INSTRUCTION: Please tick (√) the correct options besides each question and also fill in the spaces provided where appropriate with the correct options.

1. What is your Age: (a) 15-20 [ ] (b) 21-25 (c) 26-30 [ ] (d) 31-40 [ ] (d) 41-45 [ ]
2. Religion: (a) Christianity [ ] (b) Muslim [ ] (c) Traditional [ ] (d) Others (Please Specify) ……..
3. Ethnicity (a) Igbo [ ] (b) Hausa [ ] (c) Yoruba [ ] (d) Fulani [ ] (e) Others (please specify)……
4. Marital status (a) Married [ ] (b.) Single [ ] (c.) Separated [ ] (d) Widowed [ ]
5. Number of children (Parity) (a) None [ ] (b) 1-2 [ ] (c) 3-5 [ ] (d) above 5 [ ]
6. Education level (a.) No formal education [ ] (b.) Primary [ ] (c.) Secondary [ ] (d.) Tertiary [ ] (e) others (specify)……
7. Your occupation: (a) Artisan e.g Carpenter, Hairdresser, Tailor, Driver [ ] (b) Civil servant e.g Teacher [ ] (c) Self-employed e.g Trader, Photographer [ ] (d) Unemployed [ ] (e) Professionals e.g. Doctor, Nurse, Lawyer, Accountant [ ] (f) Others (please specify)_____
8. What is your Level of Income (a.) 1-10,000 [ ] (b.) 11,000-30,000 [ ] (c.) 31,000-50,000 [ ] (d.) 51,000-100,000 [ ] (e) above 100,000 [ ]

SECTION B: KNOWLEDGE OF LONG LASTING INSECTICIDE-TREATED NETS

Please tick (√) the correct options besides each question and also fill in the spaces provided where appropriate with the correct options.

9. Have you heard about Malaria (a) Yes [ ] (b) No [ ]
10. Have you suffered from Malaria before? (a) Yes [ ] (b) No [ ]
11. What causes malaria (a) Germs [ ] (b) Dirts/stagnant water [ ] (c) Mosquito bites [ ] (d) plasmodium organisms [ ] (d) others……..

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12. How is malaria Transmitted? (a) Bites of any Mosquito [ ] (b) Bites of insect which has bitten a malaria Patient [ ] (c) Stagnant water and unclean environment [ ]
13. Have you heard about Long Lasting Insecticide treated Nets (LLITNs)? (a) Yes [ ] (b) No[ ]
14. How did you hear about it? (a) Health center [ ] (b) Media [ ] (c) Friends/Family [ ] (d) Publications/Journals [ ] (e) School [ ] (f) others……
15. Long Lasting Insecticide treated Nets (LLITNs) is Key in Prevention of Malaria due to its Durability (a) Yes [ ] (b) No [ ]
16. Long Lasting Insecticide treated Nets (LLITNs) is effective in the Prevention of Malaria when it is air dried frequently (a) Yes [ ] (b) No [ ]

SECTION C: DISTRIBUTION AND OWNERSHIP OF LONG LASTING INSECTICIDE- TREATED NETS AMONG RESPONDENTS

17. Has Insecticide Treated Nets been distributed in your Area? (a) Yes [ ] (b) No [ ]
18. Do you have any Insecticide Treated Net? (a) Yes [ ] (b) No [ ]
19. Do you Own any Long Lasting Insecticide treated net(a) Yes [ ] (b) No [ ]
20. How did you get it? a) Health center [ ] (b) Market [ ] (c) A Friend [ ] (d) others……
21. How Many ITNs do your household Own? (a) None [ ] (b) 1 [ ] (c) 2-3 [ ] (d) 4 [ ] (e) above 4 [ ]

SECTION D: LEVEL OF UTILIZATION OF LONG LASTING INSECTICIDE-TREATED NETS

Please tick (√) the correct options besides each question and also fill in the spaces provided where appropriate with the correct options.

22. Have you Ever Slept under an LLITN? (a) Yes [ ] (b) No [ ]
23. Did you sleep under an LLITN Last Night? (a) Yes [ ] (b) No [ ]
24. If No, when was the last time you slept under an LLITN? (a) <7days ago [ ] (b) 8-29days [ ] (c) > 30 days [ ]
25. Do your children/family Members sleep under LLITN? (a) Yes [ ] (b) No [ ]
26. Did they sleep under an LLITN last Night?
27. If No, when was the last time they slept under an LLITN? (a) <7days ago [ ] (b) 8-29days [ ] (c) > 30 days [ ]

SECTION E: FACTORS INFLUENCING THE COVERAGE OF LONG LASTING INSECTICIDE- TREATED NETS

Please tick (√) the correct options that influence Utility and coverage of Long lasting Ins ecticide treated Nets in the spaces provided in the Table below.

S/N	Factors Influencing Coverage	Yes	No
1.	Nets Inflicts Rashes		
2.	Distance to Facility		
3.	Cultural Acceptance		
4.	Family Factors		
5.	Religious Acceptance/Factors		
6.	Difficulty to Hang		
7.	Have Door Nets		
8.	Information during Distribution		

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Perspective Article: Challenges toward Developing African Swine Fever Virus Vaccine

By Erfan Ahmed

Introduction- African swine fever virus is one of the most common viruses that infect swine population. This virus causes a systemic disease to swine named African swine fever. The virus was first identified in Africa in 1921 (Montgomary, 1921). African swine fever virus is a member of the Asfarviridae family, and it is the only DNA virus within that family (Costard et al., 2013). Though the virus is endemic to Africa, the virus has been reported to infect swine population in European countries as well, most prominently in the Caucasus region and Russian Federation. African swine fever virus is highly contagious and can lead to 100% morbidity (Costard et al., 2013). The mortality rate due to the disease is varying. The transmission cycle of the virus can continue with or without the presence of the vector (NAHIS). The usual vector in the transmission cycle is the soft ticks of the Ornithodoros species (Guinat et al., 2016). The natural host of the virus is domesticated pigs, wild boar, bush pigs, warthogs, and giant forest hogs. Vector-mediated transmission occurs through the bites of *Ornithodoros* spp. soft ticks. These are bloodsucking ticks that can transmit the infectious agent from one host to another.

GJMR-F Classification: NLMC Code: QY 60.S8

PERSPECTIVEARTICLECHALLENGESTOWARDDEVELOPINGAFRICANSWINEFEVERVIRUSVACCINE

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Perspective Article: Challenges toward Developing African Swine Fever Virus Vaccine

Erfan Ahmed

I. Introduction

African swine fever virus is one of the most common viruses that infect swine population. This virus causes a systemic disease to swine named African swine fever. The virus was first identified in Africa in 1921 (Montgomary, 1921). African swine fever virus is a member of the Asfarviridae family, and it is the only DNA virus within that family (Costard et al., 2013). Though the virus is endemic to Africa, the virus has been reported to infect swine population in European countries as well, most prominently in the Caucasus region and Russian Federation. African swine fever virus is highly contagious and can lead to 100% morbidity (Costard et al., 2013). The mortality rate due to the disease is varying. The transmission cycle of the virus can continue with or without the presence of the vector (NAHIS). The usual vector in the transmission cycle is the soft ticks of the *Ornithodoros* species (Guinat et al., 2016). The natural host of the virus is domesticated pigs, wild boar, bush pigs, warthogs, and giant forest hogs. Vector-mediated transmission occurs through the bites of *Ornithodoros* spp. soft ticks. These are bloodsucking ticks that can transmit the infectious agent from one host to another. The direct method of infectious agent transmission occurs primarily through the nosocomial secretion. The virus mainly enters the body through the upper respiratory tract. The virus has been found in all secretion and excretion of infected domesticated pig (NAHIS). Direct contact with an infected agent containing secretion from an infected animal transmit the disease to another susceptible host. Also, the virus can remain in tissue and blood for a certain period. consumption of tissue of infected animal can spread the disease readily (Guinatet al., 2016). Once exposed, the pigs start showing the symptom within 3 to 7 days (NAHIS). The symptom is mainly systemic (e.g., fever, anorexia, lethargy, etc.). Also, a sign of hemorrhagic lesion may occur on the skin. Bloody diarrhea has also, been reported in cases (NAHIS). However, the treatment option for the disease is very limited. Most importantly, no effective vaccine has been developed for the virus. In this article, the challenges toward developing a definitive vaccine for African swine fever virus will be discussed.

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II. Immune Response for Disease Protection

Though the disease is a burden to the pig population in several parts of the world, the immune response required for the protection from this virus has not yet been understood properly. In a broad understanding, both innate and adaptive immune response is necessary for the protection from the African swine fever virus. However, the cause of the high morbidity due to the disease pertains to the fact that the virus can modulate both innate and adaptive immune response of the host. The African swine fever virus attacks the macrophage, monocyte and dendritic cell for replication (Sánchez-Cordón et al., 2018). It predominantly attacks macrophages which is a key factor for both adaptive and innate immune response. Attacking the macrophages enable the virus to manipulate these two types of response. When a virus enters the macrophage for replication, the macrophage challenges the replication with the oxidizing environment. This oxidizing environment cause disruption in the DNA structure. African Swine fever virus acquired the adaption to replicate in the environment by obtaining a base excision DNA repair system (Reis et al., 2016). Also, the virus encodes a protein that can inhibit the transcription of host defense protein. The protein inhibits the transcription of interferon, chemokines, cytokines, and adhesion molecule through producing multiple proteins. This virus also inhibits programmed cell death by the host. It produces four protein that can inhibit the signaling pathway for apoptosis, which is an important phase is necessary for both innate and adaptive immunity. These unique mechanisms of interfering various cellular pathway for the development of immune response enable the virus to exploit the innate and adaptive immune system of the host and replicate rapidly to develop a highly contagious disease.

III. Current Disease Control Strategies

Currently, there is no definitive treatment for this disease. Also, no vaccine has been developed yet to prevent disease occurrence. So, the current control measure is based on the rapid diagnosis of the infected animal and slaughtering them. Appropriate disposal after slaughtering is an important step to prevent further transmission to other susceptible hosts. So, the

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preventive measure is at the forefront of controlling African swine fever virus transmission. The preventive measure is determined based on the current disease knowledge and the epidemiology of the disease. The government and international organizations are working together in the endemic area to develop the disease surveillance system for early diagnosis and prevent disease transmission. In African endemic areas, pig sector stakeholders are being informed about the disease. Ongoing programs are creating awareness in the pig farmers about the ways of prevention. Early detection of disease, management, and emergency response system is being strengthened by initiating multisectoral collaboration. However, the disease monitoring and surveillance system are still weak in that region, which making the disease elimination more difficult in that region (Gallardo et al, 2013). In the European endemic region, along with above-mentioned measure they have introduced some new measure including strict control during a border crossing, Increasing the biosecurity on farms and livestock markets. These methods helped the European countries to contain the disease in a specific part of the region. The current control program in Eastern Europe is an ideal one to ensure the eradication of the virus from that region (Gallardo et al, 2013).

IV. The Current Approach to Vaccine Development

Successor vaccine development against a microorganism requires complete knowledge about the immune response in the host body following exposure to that organism. In the case of African swine fever virus, the complete knowledge about the immune response in the host body has not yet been understood properly. It is known that the affected pig develops some immunity against the virus following recovery from the virus (Aries et al., 2017). However, it was discussed in the previous section that the virus can modulate the host immune system by inhibiting multiple signaling pathways which is important in orchestrating immune response. Development of a vaccine is largely dependent on identifying the key genes and proteins that play a central role toward evasion of the host immune system. Some progress towards the characterization of such virus "host evasion" genes has been made (Aries et al., 2017). Till date, multiple approaches for developing a vaccine against the virus has been taken. Inactivated viruses, recombinant proteins/peptides, viral vectors for antigen delivery, and live-attenuated vaccines have experimented (Reville et al., 2017). But noteworthy success is yet to come. In the next section, some of the effort to develop a live attenuated vaccine inactivated vaccine and subunit vaccine will be discussed.

a) Inactivated vaccine

Efforts to develop a vaccine using inactivated infected cell extracts supernatants of infected pig peripheral blood leukocytes, purified and inactivated virions, infected glutaraldehyde-fixed macrophages, and detergent-treated, infected alveolar macrophage cell cultures have been made previously. Also, inactivated virus strain was tried to use with efficient adjuvants. However, these efforts have failed to render the idea of formulating an inactivated African swine fever virus vaccine most unlikely (D.L. Rock, 2016).

b) Subunit vaccine

It was discussed earlier that African swine fever virus encodes multiple proteins to invade host immune system which makes the task of selecting candidate antigens for developing subunit vaccines very difficult. Aries et al., 2017 described that immunization of pigs with the protein expressed in baculovirus p54 and p30 showed significant improvement. However, the combination of p54 + p30 + p72 baculovirus proteins failed to protect the host. Also, DNA vaccines encoding p54 and p30 could not induce neutralizing antibodies. Immunizing pigs with a gene fusion of p30/CP204L and p54/E183L could not protect the host when challenged with a virulent strain. A fusion of three African swine fever virus genes (CD2v/EP402R, p54/E183L, and p30/CP204L) to the ubiquitin gene was able to produce partial protection. But the protection was not enough to produce the required antibody.

c) Live Attenuated vaccine

Reville et al., 2017 discussed the efforts of developing a live attenuated viral vaccine. According to the article, the first attempt used attenuated African Swine Fever Virus strains OURT88/3 and NH/P68. These two attenuated strains protected pigs against homologous virulent strains but gave only partial cross-protection against heterologous viruses. Moreover, excessive side effects were noticed in the post-vaccination period. Genetically modified NH/P68 strain was also tried but it failed to provide 100% protection against both homologous and heterologous virus. A recombinant strain of the virus (modified Georgia 2007) also could not protect the pigs from infection. Recently, attenuated African swine fever virus strains like Benin, Georgia, OUR/88/1, and Ba71 have shown promising efficacy toward certain heterologous and homologous virus.

V. Challenges for Vaccine Development

It has been almost 100 years since the identification of African Swine fever virus. However, it is a matter of disappointment that the detailed pathogenesis is still unknown for this virus. There are gaps in knowledge of host resistance mechanism and the viral target receptor. To develop a successful vaccine,

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require detail knowledge in these areas. These knowledge gaps are acting as a barrier to the development of an efficacious vaccine. Although Multiple vaccines have experimented, they failed to provide complete protection against all virulent strains. Live attenuated vaccine is showing promising effectiveness against some virulent strain. But to ensure 100% efficacy, detail studies are required on the mechanism of the virus' ability to modulate host immune responses. Some of the live attenuated vaccines failed to protect against the heterologous virus. Identification of cross-protective epitopes on virus protein can solve the mystery behind this failure. Live attenuated vaccine or DNA vaccine were noticed to provide partial protection. The reason behind failure to provide full protection can be identified by complete knowledge of humoral and cellular response on host body cells. Some research shows that bush pig and warthog have some resistance ability to the virus. Detailed knowledge of that resistance mechanism can help to identify the receptor of the virus on the host cell. This knowledge will eventually lead to a selection of successful vaccine receptor. However, one of the limitations is the lack of availability of wild pig species for experimentation. Some Subunit vaccine has shown efficacy toward certain virulent strain. So, further, emphasize should be given to adding antigens to those vaccines so that they can achieve full protection. Knowledge of protective antigen is needed for this task.

VI. Conclusion

African swine fever virus has been endemic to Africa and some region of Eastern Europe. But there is potential to spread the disease in other parts. The disease itself is a huge economic burden on the pig industry. An outbreak of African swine fever can cause US$910,836.70 loss in a single year (Fasina et al., 2011). An effective way to prevent the disease outbreak is developing an effective vaccine. Lately, several types of vaccine have experimented including inactivated viruses, recombinant proteins/peptides, DNA vaccines and live-attenuated vaccine (LAV). Unfortunately, none of the candidates has proven efficacious to date. However, the hope of developing a successful virus is high. A better knowledge of virus structural protein, the gene involved in the host evasion mechanism and host immune response following exposure is needed for the success to the path of developing an effective vaccine for African swine fever virus.

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Color charges: Authors are advised to pay the full cost for the reproduction of their color artwork. Hence, please note that if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a Color Work Agreement form before your paper can be published. Also, you can email your editor to remove the color fee after acceptance of the paper.

Tips for writing a good quality Medical Research Paper

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

4. Use of computer is recommended: As you are doing research in the field of medical research then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

5. Use the internet for help: An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.

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6. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right? It is a good habit which helps to not lose your continuity. You should always use bookmarks while searching on the internet also, which will make your search easier.

7. Revise what you wrote: When you write anything, always read it, summarize it, and then finalize it.

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

9. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

10. Use proper verb tense: Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. Adding unnecessary information: Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. Upon conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear: Adhere to recommended page limits.

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Mistakes to avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

Approach:

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

Procedures (methods and materials):

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.

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Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."

Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

The Administration Rules

Administration Rules to Be Strictly Followed before Submitting Your Research Paper to Global Journals Inc.

Please read the following rules and regulations carefully before submitting your research paper to Global Journals Inc. to avoid rejection.

Segment draft and final research paper: You have to strictly follow the template of a research paper, failing which your paper may get rejected. You are expected to write each part of the paper wholly on your own. The peer reviewers need to identify your own perspective of the concepts in your own terms. Please do not extract straight from any other source, and do not rephrase someone else's analysis. Do not allow anyone else to proofread your manuscript.

Written material: You may discuss this with your guides and key sources. Do not copy anyone else's paper, even if this is only imitation, otherwise it will be rejected on the grounds of plagiarism, which is illegal. Various methods to avoid plagiarism are strictly applied by us to every paper, and, if found guilty, you may be blacklisted, which could affect your career adversely. To guard yourself and others from possible illegal use, please do not permit anyone to use or even read your paper and file.

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Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals.

Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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