Seroprevalence of Influenza A/H1N1 virus among patient with Chronic Kidney Disease in North-Eastern Nigeria.

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Abstract

Introduction: Influenza A H1N1 infection can cause respiratory and multiple organ failure, which can lead to fatality. The prevalence of circulating Influenza A H1N1 viral IgG antibodies among patients with Chronic Kidney Disease (CKD) is not well understood. This study aimed to investigate the prevalence of circulating Influenza A H1N1 viral IgG antibodies among patients with CKD.

Methods: The study was conducted at the University of Maiduguri Teaching Hospital (UMTH) from January to November 2020. Ethical approval was obtained from the University of Maiduguri Teaching Hospital Ethical Committee, and informed consent was obtained from participants. Patients with a Glomerular Filtration Rate (GFR) of less than or equal to 90mL/min with associated CKD risk factors and proteinuria were included, while patients with a GFR of 90 mL/min without CKD risk factors and patients with a GFR greater than 90 mL/min were excluded. One hundred and sixty consented subjects had their blood samples collected and analyzed for H1N1 IgG antibodies using ELISA technique. Other sociodemographic and health information were obtained from patient health records. Data analysis was carried out using the statistical package for social sciences (SPSSTM) version 20.0.

Results: Out of 160 CKD blood samples tested, 23 (14.4%) were seropositive for circulating influenza A (H1N1) IgG antibodies. The seroprevalence of influenza A/H1N1 circulating antibodies was higher in the age groups of 35-44 years and 55-64 years (8 or 34.8%). The seroprevalence was higher among females (17 or 73.9%) than males (6 or 26.1%). Most of the study subjects had a normal body mass index (10 or 43.5%). Chronic Kidney Disease stage 3 patients had the highest number of patients with circulating H1N1 antibodies (10 or 43.5%) with a P value > 0.594.

Conclusion: This study suggests that H1N1 virus may be considered as an additional risk factor in CKD patients, particularly CKD Stage 5 - End Stage Renal Disease

Keywords: Influenza Virus, H1N1, Seroprevalence, Chronic Kidney Disease.

Running title*: H1N1 Virus seroprevalence Among* CKD *Patients Attending UMTH-Nigeria*

Introduction

Influenza A/H1N1 led to the infection of about 500 million people and death of almost 100 million people worldwide in the 1918 pandemic. The swine flu variant of the H1N1 which is the descendant of the 1918 Influenza A/H1N1 and also known to infect man contributes to the common seasonal influenza pandemic every year (Nickol and Kindrachuk, 2019). Although the morbidity and mortality repercussion of Influenza virus is underrated in Nigeria (Aishatu et al., 2019), the virus can lead to severe health complications likerespiratory failure and hemodynamic instability and in some cases multiple organ failure related to systemic inflammatory response, hypoxia, and direct cytopathic effect of the virus(CDC, 2022). Influenza infection often associated with end-stage renal disease to serious morbidity and mortality and there is evidence of Influenza A/H1N1 virus identification from the kidney of patients who died of Chronic Kidney Disease.

Chronic Kidney Disease is more frequent in patients with severe H1N1, with an incidence of around 50 - 60%, being associated with hemodynamic instability, advanced age and rhabdomyolysis. H1N1 virus has been shown in urine and in kidney cells of renal transplant patients(Bagshaw *et al.,* 2013).Chronic Kidney Disease patients have significant immune dysregulation as compared with the general population and subsequently have a high susceptibility to infection and a poorer response to vaccination.

Mankind and animal have both suffered from H1N1 infection yet to the best of our knowledge, there is currently no literature report on prevalence of influenza A/H1N1 viral infection among Chronic kidney disease. Little information about the presentation and clinical course of the 2009 pandemic influenza A/H1N1 virus infection in Chronic Kidney Disease is available in the literature. Thus, the main aim of the studies is to determine the seroprevalence of H1N1 amongChronic Kidney Disease patients by determining circulating H1N1 viral IgG antibodies among subjects with Chronic Kidney Disease in the study area.

Materials and Methods

Study Area

The study was carried out at University of Maiduguri Teaching Hospital (UMTH) located in Maiduguri, Borno State in North-East Nigeria (Wikipedia, 2020). It lays Latitude 11° 49' 59.99" N and Longitude 13° 08' 60.00" E with a population of 1,907,600. Located within latitude 10 N and 14 N and longitude 11 30 E and 14 45 E, Borno State which has an area of 61,435 sq. km shares borders with Republic of Niger to the north, Republic of Chad to the Northeast and Cameroon Republic to the East. It also shares border with Adamawa State to the South, Gombe State to the southwest and Yobe State to the West (Google earth, 2012).

Study Design

The study is a cross sectional studies carried out from January to November 2020. Blood samples were collected into a labeled container, separated and stored for the study at the Immunology Department of UMTH for analysis using ELISA technique. Consent forms and questionnaires were given to patients with Glomerular Filtration Rate (GFR) of less than or equal to 90mL/min/1.73m^2 with associated Chronic Kidney Disease risk factors and proteinuria and were recruited for the study.

Sample Size Determination

The minimum number of subjects that will participate in the study was determined by the formulae as previously described by CharanLwanga and Lemesho (1991)

$$n = \frac{Z^2 pq}{d^2}$$

Where:

n= Minimum Sample Size of subjects required for the study.

Z= statistic for a level of 95% confidence interval = 1.96

d= level of precision = 0.05

p = prevalence rate of Influenza A/H1N1 = 0.103 (Kate *et al.,* 2011).

q = 1-p = 0.895

$$n = \frac{1.96^2 * 0.103 * 0.895}{0.05^2}$$

$$n = 141.65516$$

According to a study by Kate *et al.*(2011), the global prevalence of Influenza A/H1N1is 10.3%, the minimum number of subjects in the cases was calculated as 141.6. However, a total of 160 subjects were enrolled into the study.

Sampling Technique

The sampling technique used was Convenient Sampling.

Method of Data Collection

Patients with Glomerular Filtration Rate (GFR) of less than or equal to 90mL/min/1.73m^2 with associated Chronic Kidney Disease risk factors as well as proteinuria were recruited in the study,

Thesocio-demographic details and Information on medical history of the patients was obtained from their hospital medical records such as medical history, provisional diagnosis, clinical and other laboratory findings result

Sample Collection, Preparation and Separation.

Two millilitres of whole blood were collected from individual Chronic Kidney Disease patients using standard venipuncture phlebotomy (Wayne, 2003). and dispensed into a plain-tube, allowed to clot for 30 minutes and later centrifuged at 5000 rpm for 10 minutes to obtain clear serum which was then transferred into cryovial and stored at -70°C before laboratory analysis. The refrigerated serum was used for detecting IgG antibodies against H1N1 influenza virus by Enzyme Linked Immunosorbent Assay.

Ethical Consideration

Ethical approval for the studies was obtained fromresearch and ethics committee of the University of Maiduguri Teaching Hospital approved the study, with this reference (ADM/TH/497/VOL.I) and approval number: (UMTH/REC/18/356)

Informed consent was sought for and obtained prior to enrollment of participantswho met the study criteria. All participants' rights were observed and bear no financial burden. Participants' data were treated with the utmost

confidentiality in accordance with the standards of human experiments and the Helsinki Declaration of 1975, as revised in 2013 (WMA, 2013).

Laboratory Analysis

Enzyme Linked Immunosorbent Assay:

Enzyme Linked Immunosorbent Assay (ELISA)kit obtainedwas manufactured by Melsin Medical Co., Limited, LOT Number: P20191023, Manufacturing Date: Oct. 23rd. 2019, Expiry date: April. 22nd. 2020. Samples were analyzed for the presence of IgG antibodies against influenza, H1N1 using this ELISA kit according to manufacturer's instruction.

Data Analysis

The data obtained from questionnaire administered were analyzed using the statistical package for social sciences (SPSS™) version 20.0 Chicago IL, USA, computer software. Statistical significance correlation was evaluated using Pearson's correlation test. P value < 0.05 was considered statistically significant.

Results

Distribution of Socio-demographic Characteristics among Chronic Kidney Disease Patients

The prevalence of Chronic Kidney Disease in respect to age group (Table 1) was found to be higher among the age group 55-64 years 41(25.6%) and lowest was among 15-24 years 4(2.5%) compared with other age group cross the subjects. Female subjects were found to be higher 114(71.2%) than their male counterpart with 46(28.8%). Most of the study subjects have tertiary education 91(56.9%) while subjects with primary Education were the lowest 11(6.9%). Most of the study subject had normal body mass index93(58.1%), and while the obese counterpart had the lowest frequency of the recruited subjects 10(6.3%) at $p \geq 0.05$

Table 1: Distribution of Sociodemographic Characteristics among Chronic Kidney Disease Patients

Variable	Frequency	Percent
Age Range		
15-24 years	4	2.5

25-34 years	14	8.8
35-44 years	45	28.1
45-54 years	37	23.1
55-64 years	41	25.6
65 and above years	19	11.9
Total	**160**	**100.0**
Gender		
Male	46	28.8
Female	114	71.3
Total	**160**	**100.0**
Educational Status		
Primary	11	6.9
Secondary	25	15.6
Tertiary	91	56.9
None	33	20.6
Total	**160**	**100.0**
Body Mass Index		
Normal	93	58.1
Under weight	28	17.5
Over weight	29	18.1
Obese	10	6.3
Total	**160**	**100.0**

Influenza A (H1N1) IgG Seroprevalence Among Chronic Kidney Disease Patients

Out of the 160 blood sample analyzed, 23(14.4%) have shown evidence of circulating influenza A (H1N1) IgG antibodies, with no significant different with P value =0.867 using chi square x^2 (1) = 0. 028 as shown in (Table 2) below.

Table 2: Influenza A (H1N1) IgG Seroprevalence Among Chronic Kidney Disease Patients

Total No. Tested	No (%)IgG Positive	No (%)IgG Negative
160	23 (14.4)	137 (85.6)

Sociodemographic Characteristics Among Chronic Kidney Disease PatientswithH1N1

The seroprevalence of H1N1 among Chronic Kidney Disease in respect to age group (Table 3), H1N1 circulating antibody has bimodal age distribution for both higher and lower age groups as 35-44 and 55-64 years 8(34.8%) and lowest among 15-24 and 65 and above years 1(4.3%) compared with other age group among the subjects. The seroprevalence of H1N1 among Chronic Kidney Disease patients regarding gender showed high frequency of H1N1 circulating antibody of 17(73.9%) among femalesand6(26.1%) inmales. Body Mass Index individuals showed have high circulating H1N1 IgG antibodies 10(43.5%) among the study demographic with$p \geq 0.05$ which showed no significance.

Table 3: Sociodemographic Characteristics Among Chronic Kidney Disease Patientswith H1N1

Variable	Frequency (%)	Percentage
Age Range		
15-24 years	1	4.3
25-34 years	2	8.7
35-44 years	8	34.8
45-54 years	3	13.0
55-64 years	8	34.8
65 and above years	1	4.3
Total	**23**	**100**
Gender		
Male	6	26.1
Female	17	73.9

Total	**23**	**100**
Primary	1	4.3
Secondary	3	13.0
Tertiary	15	65.2
None	4	17.5
Total	**23**	**100.0**
Body Mass Index		
Normal	10	43.5
Under weight	6	26.1
Over weight	3	13.0
Obese	4	17.4
Total	**23**	**100.0**

Prevalence of Circulating H1N1 antibodies among Chronic Kidney DiseasePatients Based on GFR.

With regards to Chronic Kidney Disease stage distribution, the incidence of H1N1 circulating antibodies was found to be high among stage 3 Chronic Kidney Disease patients 10(43.5%) and low on stage 5 Chronic Kidney Disease 5(21.7%), *P value* of 0.594 using Chi square.

Table 4: Prevalence of Circulating H1N1 antibodies among Chronic Kidney DiseasePatients Based on GFR.

Chronic Kidney Disease **Stages**	**Frequency (%) positive**	**Frequency (%) Negative**	**P value**
Stage 1-Normal or High GFR			0.594
Stage 2-Mild Chronic Kidney Disease			
Stage 3-Moderate Chronic Kidney Disease	10(43.5)	44 (32.1)	
Stage 4-Severe Chronic Kidney Disease	8 (34.8)	51 (37.2)	

Stage 5-End Stage Chronic Kidney Disease	5 (21.7)	42 (30.7)
Total	**23 (100)**	**137 (100)**

Discussion

This studyinvestigates Influenza A H1N1 virus as a risk factor for Chronic Kidney Disease by determining its seroprevalence amongst Chronic Kidney Disease selected subjects, and from the study, the frequency of Chronic Kidney Diseasewas higher in age group 55-64 years 41(25.6%), this may be due to the progressive decrease in glomerular filtration rate (GFR) with age which similar Rainer Düsing, (2012) who reported a progressive decrease in glomerular filtration rate (GFR) as age increases with greater percentage of individuals older than 65 years. Distribution of Chronic Kidney Diseasewas found to be higher among females 114(71.2%) than their male counterpart with 46(28.8) this may not be unconnected with increase response to female gonadotropic hormone(Jafri *et al.,* 2015) related to the fact that pregnancy can also increase the risk of kidney disease such as pre-eclampsia occurring in 3 to 10% of pregnancies and is associated with acute kidney injury (AKI), increased risk for hypertension as well as Chronic Kidney Disease".

The frequency of subjects with tertiary education was higher at 91(56.9%) and lowest amongsubjects with primary Education at 11(6.9%) possibly due to awareness and knowledge about Chronic Kidney Disease acquired through various means during the trail of acquiring especially the tertiary education and early hospital visit where those with only primary education may not have much idea if at all they do of Chronic Kidney Disease. Chronic Kidney Disease, has higher frequency among subjects with normal Body Mass Index 93(58.1%), and lower among obese 10(6.3%) at $p \geq 0.05$ and this according to the information obtained in the patient's records, patients with either normal or underweight Body Mass Index have been hospitalized for long and have undergone wasting.

Of the 160 serum samples analysed, 23(14.4%) have shown evidence of circulating influenza A (H1N1) IgG antibodies, the findings agree closely with the that of Watanabe, (2013) who reported Influenza A(H1N1)pdm09 virus presence in Kidney of patients with Acute Kidney Infection (AKI) patients and present clinically with rhabdomyolysis associated

with influenza.Similarly, Li and Wang, (2010) reported "Acute tubular necrosis present in all subjects involved in the study and H1N1 viruses found in the cytoplasm of glomerular macrophages in the kidneys of 80% of patients.

The seroprevalence of H1N1 among Chronic Kidney Disease in respect to age group, H1N1 circulating antibody was found to be higher among age group 35-44 and 55-64 years 8(34.8%) and lowest among 15-24 and 65 and above years 1(4.3%), This is because the young and old people tend to have very low immunity than people in the middle age group. The seroprevalence of H1N1 among Chronic Kidney Disease patients regarding gender showed high frequency of H1N1 circulating antibody of 17(73.9%) among females and 6(26.1%) in males this has been linked to hormonal and gonadotropic changes in women.

Body Mass Indexindividuals showed high circulating H1N1 IgG antibodies 10(43.5%) among normal subject and lower among overweight 3 (13.0%). This could be because some of the patients a hospitalized for long and may have undergone wasting.

The current studies shows the incidence of H1N1 circulating antibodies to be high among stage 3 Chronic Kidney Disease patients 10(43.5%) and low on stage 5 Chronic Kidney Disease 5(21.7%), which is similar to the findings ofEleftheriadis, (2007) who reported "Chronic Kidney Disease Patients and End Stage Renal Disease on the replacement therapy patients have significant immune dysregulation as compared with the general population and, subsequently, have a high susceptibility to infection increased levels of cardiovascular disease".

The lower seroprevalence of H1N1 found in stage 5 (End Stage Chronic Kidney Disease), 5 (21.7%), which indicates decrease in circulating H1N1 specific IgG antibody as Chronic Kidney Disease progresses. Although we did not come across any work that related Influenza virus infection and Chronic Kidney Disease stages, this finding can be related to the fact that once Chronic Kidney Disease has reached the End Stage, the kidney loses most of its function including its ability to participate in antibody production.

Conclusion

There is an evidence of circulating H1N1 specific IgG antibody in Chronic Kidney Disease and its mostly at Stage 3-Moderate Chronic Kidney Disease.

Limitation of the Study

The most important limitation of this study is financial constraints which did not allow for enrolment of very large sample.

Strength of the study

The strength of the study is the ability of the research to determine IgG antibody which may indicate that an individual has atleast gotten exposed to the virus at a point in time.

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Conflict of Interest

The authors declare that **there is no** conflict of interest regarding the publication of this manuscript.

Authors' role and contribution:

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Bamidele Oderinde Soji; clinical studies of patients and mobilization, definition of intellectual contents.

Inuwa Yahaya; manuscript review and data analysis

Isa Aliyu Ibrahim; design of manuscript and manuscript review

Liman Muhammad Bashir; Assay sample for ELISA technique.

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Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on request.

References

Aishatu, B. G., Saka, J. M., Aisha, M., Ado, Z., Oladayo, B. (2013). Co-morbidity Factors Associated with Influenza in Nigeria; Online J Public Health Inform: 5(1): e110. PMCID: PMC3692755

Bagshaw, S. M., Sood M. M., Long, J., Fowler, R. A., Adhikari, N. K. (2013). Canadian Critical Care Trials Group H1N1 Collaborative. Acute kidney injury among

critically ill patients with pandemic H1N1 influenza A in Canada: cohort study. BMC Nephrol. 14, 123.

Centre for Disease Control and Prevention. (2022). Flu symptoms and complications. National Centre for Immunization and Respiratory Diseases.

Eleftheriadis, T., Antoniadi, G., Liakopoulos, V., Kartsios, C., Stefanidis, I. (2007). Disturbances of acquired immunity in hemodialysis patients. Seminars in Dialysis. 20(5):440–451.

Encyclopedia Britannica. (2007). Retrieved 6 April, 2007.

Google Earth, (2012), coordinates of Maiduguri.

Jafri Z, Bhardwaj A, Sawai W and Sultan N. (2015) Influence of Female Sex Hormones On Periodontium: A Case Series. J. Nat. Sci. Biol. Med. 2015; 6: S146–S149.

Kate, S., Pamela, W., Linda, J. W., Walt, E. A., Vittal, K. S., Paul, D., William, F. C., Kate, T., Fiona, C. D., Graham, M. D. (2001). Prevalence of Influenza A (H1N1) Seropositivity in Unvaccinated Healthcare Workers ins Scotland at the Height of the Global Pandemic. Journal of Environmental and Public Health, vol. 2001. Article ID 407505, 5 pages.

Li, H., Wang, S. X. (2010). Clinical features of 2009 pandemic influenza A (H1N1) virusinfection in chronic hemodialysis patients. Blood Purif. 30:172-7.

Lwanga, S. K. and Lemeshow, S. (1991). Sample Size Determination in Health Studies: A Practical Manual, Geneva: World Health Organization, 1-22.

Nickol, M. E., Kindrachuk, J. A. (2019). Year of terror and a century of reflection: perspectives on the great influenza pandemic of 1918-1919: BMC Infect Dis; (1):117.

Rainer, D. (2012). Risk Factors in the Progression of Chronic Kidney Disease. EuropeanEndocrinology. 2006(2):41-46.

Watanabe, T. (2013). Renal complications of seasonal and pandemic influenza A virusinfection. European Journal Jafri Z, Bhardwaj A, Sawai W and Sultan N. Influence of Female Sex Hormones On Periodontium: A Case Series. J. Nat. Sci. Biol. Med. 2015; 6: S146–S149.

Wayne, P. A. (2003).Procedures for the collection of diagnostic blood specimens by venipuncture. Approved standard, National

Committee for Clinical Laboratory Standard, 8(7):H3-A5.

Wikipedia. (2020). List of Nigerian states by area. Retrieved from https://en.m.wikipedia.org/wiki/List_of_Nigerian_states_by_area.

World Medical Association (WMA). (2013). World Medical Association Declaration of Helsinki ethical principles for medical research involving human subjects. Journal of American Medical Association, 310(20), 2191-2194