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Molecular Detection of Influenza A(H1N1)Pdm09 Virus Among Chronic Kidney Disease Patients: A Peripheral Blood Sample Approach and Assessment of the Associated Risk Factors

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Abstract:

Introduction: Chronic kidney disease (CKD) is a progressive loss of functional nephron characterized by various risk factors. Influenza virus has been found to cause rhabdomyolysis, which is toxic to the kidneys and can initiate or worsen CKD. This study aims to investigate the frequency and molecular detection of Influenza A(H1N1)Pdm09 Virus gene among CKD patients attending University of Maiduguri Teaching Hospital, Nigeria.

Materials and Methods: Peripheral blood samples were collected from 150 CKD patients. One-step RT-PCR was performed for detection of influenza virus using the Centers for Disease Control and Prevention protocol. Relevant clinical

data were collected in standardized questionnaires from each patient, and medical history was obtained from their hospital records.

Results: Conventional PCR analysis revealed that 16% of the CKD patients tested positive for Inf A/Pdm H1N1. The virus frequency was found to be higher among patients in CKD stage 5 (end-stage CKD) and lower in CKD stage 3 (moderate CKD). Additionally, female CKD patients and those in the age group of 55-64 years showed a higher susceptibility to Inf A/Pdm H1N1 infection.

Discussion: The study provides evidence of the presence of Inf A/Pdm H1N1 in CKD patients, aligning with previous research showing its involvement in kidney disease aggravation. CKD patients often exhibit immune dysregulation, which might facilitate the virus's invasion and persistence. Conclusion: This study provides evidence of an association between Influenza A(H1N1)Pdm09 viraemia and decreased kidney function among CKD patients. The findings highlight the importance of monitoring and preventing influenza infection in CKD patients to prevent further kidney damage.

Keywords: *Influenza A(H1N1)Pdm09, Chronic Kidney Disease, Molecular Detection, Nigeria, Immune Dysregulation.*

Introduction

Chronic kidney disease (CKD) is a condition characterized by the gradual decline and/or loss of kidney function over time. In 2016, CKD affected a staggering 753 million people worldwide, with 417 million females and 336 million males being affected (Ikbov et al., 2018). This disease imposes a significant economic burden on families. While drug-induced cases of CKD do exist, it is primarily caused by certain diseases, with diabetes mellitus, hypertension, and glomerulonephritis being the leading culprits as of 2015 (Vos et al., 2015). Shockingly, one in five adults with hypertension and one in three adults with diabetes suffer from CKD. Patients with CKD also experience significant immune dysregulation compared to the general population, rendering them more vulnerable to infections and showing poorer responses to vaccinations. Various infectious agents, such as Mycobacterium tuberculosis, leptospirosis, and more recently, Influenza A(H1N1)Pdm09 Virus (Inf A/Pdm H1N1), exploit the weakened immunity of CKD patients and have been linked to causing CKD (Yang, 2018).

Furthermore, Inf A/Pdm H1N1 has been detected in the urine and kidney cells of renal transplant patients, making it a concerning factor for individuals suffering from CKD (Bagshaw, et al., 2013). Influenza, a common and severe pathogen, poses a serious threat to patients with end-stage renal disease, leading to significant morbidity and mortality. Researchers have even identified Inf A/Pdm H1N1 in the kidneys of patients who died from Chronic Kidney Disease. This study aims to explore the frequency and molecular detection of Inf A/Pdm H1N1 among selected CKD subjects.

Materials and Methods

One hundred and fifty patients from the Nephrology Clinic at the University of Maiduguri Teaching Hospital in Nigeria were enrolled consecutively through simple random sampling. The sample size was determined using a standard formula (Lwanga and Lemeshow, 1991). The research study received approval from the Hospital's research and ethics committee (reference: ADM/TH/497/VOL.I, approval number: UMTH/REC/18/356). Informed consent was obtained from all participating patients or their guardians/relatives, especially for children or unconscious patients. The study followed the ethical standards of human experimentation outlined in the Helsinki Declaration of 1975, revised in 2003 (WMA, 2013). Participants of all age groups and both sexes with a Glomerular Filtration Rate (GFR) of less than

90ml/min/1.73 m2 were included, while those with a GFR greater than 90ml/min/1.73m2 and those who declined to give consent were excluded from the study.

The study subjects who provided consent were categorized into five groups based on CKD stages: CKD stage 1, CKD stage 2, CKD stage 3, CKD stage 4, and CKD stage 5, with the GFR/minutes serving as the criteria for grouping. Standardized questionnaires were used to collect relevant clinical data from each patient, while additional medical history information was obtained from their hospital records.

Two milliliters of blood sample were collected from each patient using standard venipuncture phlebotomy (Wayne, 2003). The blood was gently dispensed into a 5ml vacutainer tube containing two milliliters of RNA shield reagent and stored at 4°C until processing for molecular diagnosis of Inf A/Pdm H1N1 using conventional one-step reverse transcriptase PCR (WHO, 2009).

Laboratory Procedures

The RNA from all 150 collected samples was individually extracted and amplified according to the manufacturer's instructions for the AccuPrep® Viral RNA Extraction Kit by Bioneer. The purified RNA extracts then underwent one-step RT-PCR for cDNA synthesis and amplification. The amplicons were subjected to gel electrophoresis to detect the specific amplified product, and all necessary precautions were taken to minimize contamination and ensure accuracy.

Statistical Analysis

The data obtained were analyzed using the statistical package for social sciences (SPSS) version 20.0 from Chicago, IL, USA. The analysis aimed to explore the frequency of circulating Influenza virus H1N1 among CKD patients and the distribution of Inf A/Pdm H1N1 in CKD patients based on Age, Sex, Educational Status, and GFR/CKD stages.

Results

The study tested a total of 150 samples from patients with Chronic Kidney Disease. The Conventional PCR analysis revealed an overall positivity of 16% (24/150) for Inf A/Pdm H1N1 (Table 1).

Table 1. Inf A/Pdm H1N1 Frequency Among CKD Patients Based on HA Gene Detection by RT-PCR

Total No. Tested	No (%) of samples with HA gene
150	24 (16%)

Figure 1 shows the summary gel for Inf A/Pdm H1N1 HA gene detection of all 24 positive samples obtained by Conventional PCR.

Table 2 demonstrates the correlation of Inf A/Pdm H1N1 (HA) gene positivity with CKD stages. The incidence of Inf A/Pdm H1N1 circulating (HA) gene was found to be high among stage 5 CKD patients (45.8%) and low in stage 3 CKD (20.8%).

Table 2. Frequency of Inf A/Pdm H1N1 HA Gene Among CKD Patients Based on GFR/CKD Stages

CKD Stages	Frequency (%) positive	Frequency (%) Negative	P value
Stage 1-Normal or High GFR			0.594
Stage 2-Mild CKD			
Stage 3-Moderate CKD	5 (20.8)	38 (30.2)	
Stage 4-Severe CKD	8 (33.4)	47 (37.3)	
Stage 5-End Stage CKD	11 (45.8)	38 (32.5)	
Total	**24 (100)**	**1 26 (100)**	

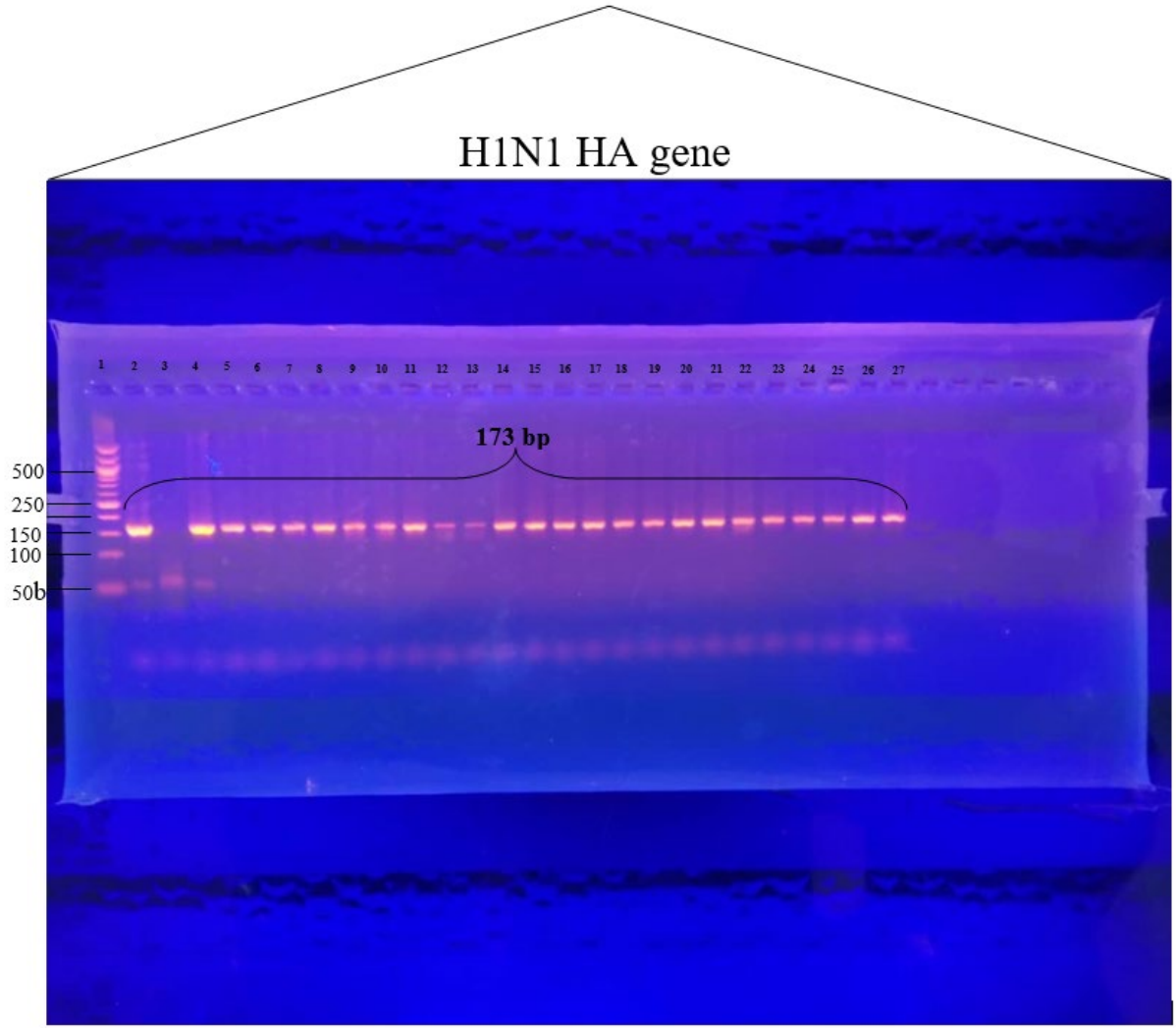

Figure 1. Summary Gel for Inf A/Pdm H1N1 HA Gene Detection of All (HA) Gene Positive Samples

Table 3 presents the socio-demographic characteristics of CKD patients based on Inf A/Pdm H1N1 (HA) gene detection. CKD patients of age group 55 – 64 were more susceptible to Inf A/Pdm H1N1 infection (37.1%) compared to age groups 15 - 24 and 55 - 64 yrs (4.2%). Additionally, Inf A/Pdm H1N1 was more common among females (70.8%) than males (29.2%). The frequency of Inf A/Pdm H1N1 was found to be high among people with tertiary education (65.2%) and very low among those with primary level education (4.3%).

Discussion

Chronic kidney disease (CKD) is a prevalent condition and a leading cause of death in the study area, often associated with hypertension, obesity, and diabetes mellitus. In contrast, Inf A/Pdm H1N1 has the potential to initiate or worsen CKD. This study highlights the possibility of detecting Inf A/Pdm H1N1 in peripheral blood samples as an alternative method of sample collection, consistent with a similar research conducted by Su-Mi et al., who detected Inf A/Pdm H1N1 gene in 11.4% of hematopoietic stem cell transplant patients' blood, suggesting that virus RNA detection in blood could serve as a marker for severity and

overall poor outcome of associated diseases (Su-Mi et al., 2012).

Table 3. Socio-Demographic Characteristics Among CKD Patients Based on Inf A/Pdm H1N1 Positivity

Variable	Frequency (%)
Age Range	
15-24 years	1(4.2)
25-34 years	2(8.3)
35-44 years	3(12.5)
45-54 years	8(33.3)
55-64 years	9(37.5)
65 and above years	1(4.2)
Total	**24(100)**
Gender	
Male	7(29.2)
Female	17(70.8)
Total	**24(100)**
Education	
Primary	1(4.2)
Secondary	3(12.5)
Tertiary	16(66.7)
None	4(16.6)
Total	**24(100)**

The frequency of Inf A/Pdm H1N1 (HA) gene among CKD patients attending the nephrology unit of the University of Maiduguri Teaching Hospital in Nigeria is 16%, which closely aligns with a study by Watanabe (2013), where Inf A/Pdm H1N1 was detected in the kidneys of some CKD patients. Watanabe (2013) further concluded that while the frequency of Inf A/Pdm H1N1 in kidney disease may not be high, it can aggravate the condition of CKD patients, consistent with the findings of Eleftheriadis et al., (2007), which highlighted the significant immune dysregulation in CKD patients and those on renal replacement therapy, making them more susceptible to infections and showing poorer responses to vaccinations.

Other studies have demonstrated an increased incidence of B cell apoptosis in patients with end-stage renal disease Patients (Fernández-Fresnedo et al., 2000) have shown increased apoptosis and reduced populations of naive and central memory T cells, as well as impaired antigen-specific memory CD4+ T cells (Litjens et al., 2008). Given that CKD patients' kidneys may not function adequately, immunosuppression could occur, as the kidneys play a vital role in immunogenicity, thereby providing an opportunity for the Inf A/Pdm H1N1 virus to thrive within these patients.

Interestingly, this study observed that CKD patients in the age group of 55-64 were more susceptible to Inf A/Pdm H1N1 infection (37.1%) compared to the age groups of 15-24 and 55-64, which showed a lower infection rate (4.2%). This finding can be attributed to the progressive decline in glomerular filtration rate (GFR) with increasing age, similar to the results reported by Rainer, (2012), who noted a higher percentage of individuals older than 60 years with a progressive decline in GFR. As kidney function decreases, patients may experience immunosuppression, making them more vulnerable to infections and less capable of producing sufficient antibodies to neutralize the virus.

Additionally, this study revealed that the frequency of Inf A/Pdm H1N1 infection was higher among female CKD patients (70.8%) compared to male patients (29.2%). This finding aligns with research that indicates females may be more susceptible to certain immune-related diseases due to sex-specific differences in immune responses (Jafri et al., 2015). Furthermore, hormonal factors and reproductive processes have been suggested to play a role in the increased susceptibility of females to viral infections, including Inf A/Pdm H1N1 (Henning et al., 2021). Understanding these sex-specific differences may be crucial in developing targeted interventions and treatments (Klein, 2012).

Moreover, the frequency of Inf A/Pdm H1N1 infection was higher among subjects with tertiary education (65.2%) and lower among those with only primary education (4.3%). This discrepancy might be due to the greater awareness and knowledge about CKD acquired during higher education, prompting individuals with higher education levels to seek early medical attention and treatment. On the other hand, individuals with primary education might lack the necessary information and may not recognize the signs and

symptoms of CKD, leading to delayed diagnosis and treatment.

Conclusion

In conclusion, this study demonstrates the involvement and association of Inf A/Pdm H1N1 with CKD, which has the potential to worsen kidney conditions. Given the significance of CKD and its high prevalence worldwide, understanding the link between CKD and Inf A/Pdm H1N1 infection becomes crucial for better management and control of the disease. The findings of this study could provide valuable insights for healthcare professionals in designing preventive and therapeutic strategies for CKD patients.

Conflict of Interest

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

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