SHORT COMMUNICATION

Assessment of Baseline Antibodies to Pandemic Influenza A/H1N1/2009 Virus in Ogbomoso, Oyo State, Nigeria

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Introduction

Acute respiratory infection remains a global leading cause of death, with influenza accounting one of the most important causes of severe infections and deaths every year [1]. Globally, an estimated 1 million persons died from respiratory and cardiovascular conditions associated with influenza A/H1N1/2009 infections.

Transmission patterns of influenza are attached with unique epidemiologic and demographic features such as environmental, economic, religious, cultural and social conditions [2]; weather conditions such as temperate and humidity; pre-existing immunity accounting for difference in the prevalence rates of specific antibodies [3].

Serum antibody to influenza can be used to identify past exposure and measure current immune status. Various laboratory methods applied to determining the host's immune response include haemagglutination inhibition (HI) test, neutralization test (NT), microneutralization (MN) test, and enzyme-linked immunosorbent assay (ELISA) [4]. Although both HI and NT tests serve as measures of antibody concentration in sera, they have important differences in how they are conducted and how they measure immunity.

Haemagglutination inhibition and NT assays, despite their widespread usage, the difference in reliability between laboratories is a direct result of how they are been measured [5]. These assays assess the level of functional immunity to a virus in a similar manner, both using serial dilution of sera applied to a fixed amount of virus to determine at which titer of sera the virus is effectively inhibited [6], however, the difference is in the biological mechanism used as an indicator for inhibition [7]. The HI assay utilizes the natural process of viral hemagglutination, a process in which a lattice forms by binding of viruses to red blood cells; this process is blocked when sufficient antibody with affinity to the virus is present [8].

The NT assay, in contrast, measures cytopathic effects of the virus, the invading and killing of cells, through plaque formation [9]. Again, the antibodies in the sample serum are tested for their ability to block this activity. Results are expressed as reciprocal of the highest dilution at which virus infection is blocked.

A more precise assessment of the total number of infected persons would require the large-scale use of techniques to specifically determine the presence and relative concentration of A/H1N1/2009 influenza virus antibodies in serum samples. Besides its obvious epidemiological significance, the availability of these techniques would also allow the rapid discernment of potentially immune subjects among those more susceptible to infection in a given population [5]. However, as a result of the somewhat time, labour intensive, specialized training, less availability of specific reagents needed for these techniques, hence, the assessment of specific baseline antibodies to A/H1N1/2009 influenza virus with an ELISA-based method for sero-epidemiological study among randomly-selected apparently healthy individuals.

The immunoassay used for this study shows significant advantages over conventional MN and HI assays; (i) it does not require fresh chicken erythrocytes; (ii) it does not require virus manipulation and therefore does not require special infrastructure; and (iii) interpretation of results rests on absorbance readings, instead of subjective visual estimation of agglutination.

Materials and methods

Study area

The study was carried out at the Bowen University Teaching Hospital situated at Ogbomoso, Oyo State in the tropical belt of Southern Western part of Nigeria (Africa). Its coordinates are 8°7'60"N and 4°16'0"E in DMS

(Degrees Minutes Seconds) or 8.13333 and 4.26667 (in decimal degrees).

Study population

Consenting apparently healthy individuals, in the age range 16–65 years, attending Bowen University Teaching Hospital at Ogbomoso were enrolled for this study between December 2014 and September 2015. Structured questionnaires about demographic characteristics and other relevant information were administered to all participating subjects as well as verbal and informed consent form prior blood sample collection. No documentation of recent travel activity was obtained in the study. The study protocol was in accordance with the amended Declaration of Helsinki.

Ethical clearance

Ethical approval for the study was obtained from the Ethics Review Board of Bowen University Teaching Hospital, Ogbomoso. Informed consent was obtained from all subjects recruited into the study.

Eligibility criteria

Any individual who refused to give consent as well as provide necessary information on the questionnaire was excluded from the study. Individuals within the age range 1–14 years were excluded from the study because their parents were not ready to give consent. Clinical history of volunteers was noted, especially blood transfusion, exposure to syringes, surgical and dental procedures. Apparently unhealthy or malnourished individuals were excluded.

Collection of blood sample

Five ml of venous blood samples were collected in tubes with no anticoagulant. The blood sample was spun in a bench centrifuge at 3000 rpm for 10 min to obtain serum. Serum was obtained and stored at -20°C until ready for analysis.

Antibody detection

Commercially available validated third generation ELISA kits (WKEA MED SURPLUS CORP, China) were employed to determine specific antibodies against influenza A (H1N1) according to the manufacturer's instructions. The Microwell plate had been coated with recombinant influenza A/H1N1 antigens. The specificity and sensitivity of the kit were 99.55% and 99.79% respectively. The optical density was read using the Emax endpoint ELISA microplate reader (Molecular Devices, California, USA) and the result was interpreted according to the manufacturer's instruction.

Table 1. Socio-demographic factors of the study participants.

Parameter	Age range	Frequency	Percentage
Age	16–25	49	26.3
	26–35	95	51.1
	36–45	32	17.2
	46–55	9	4.3
	56–65	1	0.5
Sex	Male	93	50
	Female	93	50
Marital status	Monogamy	132	71.0
	Polygamy	9	4.8
	Single	45	24.2
Occupation	Artisan	34	18.3
	Civil servant	54	29.0
	Student	42	22.6
	Trader	47	25.3
	Others	1	0.5
	None	8	4.3
Education	Primary	17	9.1
	Secondary	44	23.7
	Tertiary	106	57.0
	Others	17	9.1
	None	2	1.1

Table 2. Seroprevalence of H1N1 infections among the study participants.

Status	Total (%)
Reactive	8 (4.30)
Non-reactive	178 (95.70)
Total	186(100.00)

Statistical analysis

The prevalence of antibodies to influenza A (H1N1) was determined from the proportion of the positive individuals in the total population studied and expressed as percentages. The data obtained were analyzed using a Statistical Package for Social Sciences (SPSS version 20). They were organized and summarized in terms of frequencies and the results of the study were presented in tables. Chi-square (χ^2) test was utilized to assess the association between the socio-demographic variables and influenza A (H1N1) status. Statistical significance was set at $P < 0.05$. Logistic regression was done to determine the strength of association between the potential risk factors and influenza A (H1N1) status.

Results

The study includes 186 participants consisting of 93 males and females respectively. The participants were in the age range 16–65 years with a mean age of 31.62 ± 7.85 years. The sociodemographic characteristics of the participants are shown in Table 1. Overall, 8 (4.3%) out of 186 participants had antibodies to H1N1 (Table 2). As shown in Table 3, 6 (6.5%) of males were seropositive while 2 (2.2%) were positive for females. However, sex distribution had no statistical significance on the seroprevalence among the study individuals ($P > 0.05$). Participants in the age range 16–25 years had the highest prevalence of 10.2% compared to 3.1% and 2.1% in the age groups 36–45 and 26–35 years

Table 3. Association of H1N1 infection with sociodemographic factors.

		IgM Sero-Status						
		Reactive		Non-reactive				
Parameter	Level	Count	(%)	Count	(%)	Df	Chi-square	P-value
Age	15–25	5	10.2	44	89.8	4	5.818	0.213
	26–35	2	2.1	93	97.9			
	36–45	1	3.1	31	96.9			
	46–55	0	0.0	9	100.0			
	56–65	0	0.0	1	100.0			
Sex	Male	6	6.5	87	93.6	1	2.090	0.148
	Female	2	2.2	91	97.9			
Marital status	Monogamy	3	2.3	129	97.7	2	6.794	0.033*
	Polygamy	0	0.0	9	100.0			
	Single	5	11.1	40	88.9			
Occupation	Artisan	0	0.0	34	100.0	5	8.619	0.125
	Civil servant	1	1.9	53	98.2			
	Student	5	11.9	37	88.1			
	Traders	2	4.3	45	95.7			
	Others	0	0.0	1	100.0			
Education	None	0	0.0	8	100.0	4	1.873	0.759
	Primary	0	0.0	17	100.0			
	Secondary	1	2.3	43	97.7			
	Tertiary	6	5.7	100	94.3			
	Others	1	5.9	16	94.1			
	None	0	0.00	2	100.0			

Table 4. Association of potential risk factor and H1N1.

Variables	IgM Sero-status		OR	95% CI	p-value
	Positive N (%)	Negative N (%)			
History of blood transfusion No	8 (4.5)	171 (95.5)	0.961	0.933–0.990	0.706
Yes	0 (0.0)	7 (100.0)	1		

respectively. There was no statistically significant effect of age on H1N1 seroprevalence. Occupation wise, the highest prevalence was recorded among the students (11.9%) compared to 4.3% and 1.9% among the traders and civil servants respectively. Education wise, the highest prevalence was recorded among those that had a tertiary education (5.7%) compared to 5.9% and 2.3% among those that had other and secondary education respectively. Considering the marital status, participants who were single had a higher prevalence of 11.1% than those in the monogamy group who had a prevalence of 2.3%. There was the statistical significance of marital status on H1N1 seroprevalence ($p = 0.03$).

The possible risk factor that might be associated with acquiring H1N1 infection was analyzed (Table 4). There was no statistical association between H1N1 and the risk factor ($P > 0.05$). The result showed that 8(4.5%) of the participant with no history of blood transfusion had H1N1, however, there was no statistical significance with the infection ($\chi^2 = 0.327$, $df = 1$, $p = 0.706$). There was a 1-fold risk of getting infected among those who had the previous history of blood transfusion (95% CI = 0.933–0.990, OR = 1.0).

Discussion

In this study, the overall prevalence of antibodies against influenza A (H1N1) virus was 4.3%. The presence of the antibodies in the seropositive individuals may be as a

result of increased activities such as traveling, gatherings for social and group activities which create ideal conditions for the transmission of the virus. The recorded prevalence is lower than a 31% prevalence recorded in South Africa [10], 29% prevalence recorded in the metropolitan area of Buenos Aires of Argentina [11] and 25% prevalence recorded in Tampa Bay Florida [3]. The differences might be related to the methodological differences in the type and period of sample collection, diagnosis and differences in geographic location since factors such as overcrowding, climate, and personal hygiene, can affect the transmission efficiency of the virus [1]. Also, the variation in the epidemiology of circulating subtypes between countries might also explain the different findings in the seroprevalence studies [12].

There was a wide variation in the seropositive level against the A (H1N1) virus among the different age groups. Seroprevalence in subjects aged 15–25 years was 10.2%, 26–35 years was 2.1%, while it was 3.1% in the age range 36–45 years. The 15–25 year age group (10.2%) may be attributed to schools, churches serving as gathering places and group activity for young adults which allow conditions for the spread of the virus [13]. The 26–35 (2.1%) and 36–45 (3.1%) year age groups tend to work in air-conditioned offices, use public transport, attend meetings and social functions, as well as possibly acquired partial immunity against the influenza A (H1N1) strain. This is in agreement with the reports of Bada et al., [14], Miller et al., [15], Chen et al., [16], Tian et al., [17] and

Cox et al., [3] with higher notification rates in those adults younger than 50 years of age as the age-specific prevalence in this study is within that range. It is in contrast with other studies showing higher antibodies among elderly [15,18–20].

Also, there was a higher seroprevalence in males (6.5%) as compared with the females (2.2%). The seroprevalence was much higher in males who constitute the main group responsible for spreading the infection due to increased activities such as traveling which is common amongst males than females, gatherings for social and group activities which create ideal conditions for the transmission of the virus as compared to females in the study area which is in accordance with the report of Labato et al., [11]. Also, it can be as a result of differing social mixing patterns between and within age groups in males compared to their female counterpart. Although, there are uncertainties in measuring mixing patterns because it is not clear what type of contacts best describe the spread of influenza. However, there was no statistically significant difference in seroprevalence by sex.

Considering education-specific prevalence, the subjects that had the tertiary education (5.7%) had higher prevalence compared to other groups. This is consistent with the key role of young adults, especially in tertiary institutions, in the transmission which can be an important epidemiological determinant of community spread. This may also be explained by overcrowding in lecture halls as well as hostels for students which create an easy contraction of the virus. This observation is in accordance with the reports of Hardelid et al., [21] and Chen et al., [16].

Considering marital status, the singles (11.1%) had high antibodies against A/H1N1/2009 as compared to the other groups (monogamy and polygamy). Singles are generally involved in more social functions and movement as compared with monogamy and polygamy groups in the study area. Interestingly, antibody seroprevalence rates were statistically significant ($p = 0.033$).

Considering occupational status, students exhibited 11.9% seroprevalence. This may be explained by schools serving as gathering places for students, unhygienic conditions such as lack of or improper hand washing and good personal hygiene, the use of public transport, and religious and social meetings or functions, all of which favour transmission of the virus. This is consistent with the findings of Reed et al., [22]. Also, seropositivity was not statistically associated with the previous history of blood transfusion.

Summarily, the result revealed more prevalence amongst male tertiary students within the age range 15–25 years who were single. This result is in accordance with the transmission pattern of influenza A/H1N1/2009 which is common among young adults owing to various outdoor and indoor activities they get involved.

In conclusion, rapid diagnosis of influenza virus antibodies benefits infected individuals in allowing timely

therapy for proper prevention of complications; benefits public health to prevent outbreaks and further spread of the virus.

Authors' contribution

OEK collaborated on project conception, analyzed data and wrote the draft article, AEH and OJK collaborated on project conception, statistical analysis and helped in writing the article.

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Disclosure statement

The authors declare they have no conflicting interest.

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