Influenza, Malaria parasitaemia and Typhoid fever Coinfection in Children: Seroepidemiologic Investigation in Four Healthcare Centres in Lagos, Nigeria

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Abstract

Background : According to WHO, out of about one billion individuals usually infected with influenza virus, children from developing countries account for 99% of deaths due to related infections. There is dearth of clinical-epidemiological information on the trio of influenza, malaria, and typhoid fever co-infection in Nigeria. Similarity of their clinical symptoms coupled with lack of vaccine and all year-round circulation in sub-Saharan Africa cause serious paediatric morbidity and mortality. This study therefore investigated influenza, malaria and typhoid fever coinfection among children in Lagos, Nigeria. **Methods :** A sero-epidemiologic hospital-based study was designed. Blood from 364 children tested by RDT for malaria HRP-II/pLDH (Accessbio or Medicon BioCheck, USA) and typhoid (CTKBiotech, USA or Omega, UK) were screened by ELISA (Demeditec, Germany) for influenza virus specific IgM antibody. Descriptive statistics was used and p-values were determined with Chi-square. **Results :** Demographic data showed median age of 3 (mean 3.8, mode 2, range 0-14) years. Out of the 364 samples tested, 76/364 (20.9%) were seropositive for influenza A virus. Of the 76 seropositive patients, 47/76 (61.8%) had malaria parasitaemia, 42/76 (55.3%) had typhoid, and 21/76 (27.6%) were co-infected with malaria parasitaemia and typhoid fever. Furthermore, 2/76 (2.6%) children having underlying health condition of sickle cell anaemia recorded influenza seropositivity. Ojo primary healthcare centre had the highest seroprevalence of 48.7% (37/76), age group 1-4 years recorded the highest seroprevalence of 55.3% (42/76), the highest serologic evidence of 61.8% (47/76) was detected among male children, while fever 27.6% (21/76) was the most common of all the clinical symptoms recorded. Although, the month of March had the highest seroprevalence of 20/76 (26.3%), seropositivity was recorded in all other months considered for this study. **Conclusions :** We hereby report the first paediatric co-infection of influenza, malaria and typhoid fever with a percentage seroprevalence of 27.6% among all age group round the months. Low co-infection was however recorded in children having sickle cell anaemia. Annual vaccination is strongly recommended for children of all ages in order to prevent co-infection of influenza with other deadly diseases.

Background

Influenza, an infectious respiratory disease caused by enveloped RNA virus of the family *Orthomyxoviridae*, is largely spread via aerosols and droplets generated through coughing and sneezing [1, 2]. Annually, influenza virus infect about one billion individuals with resultant 3-5 million severe cases and 290,000 to 650,000 deaths globally [3, 4]. It affects individuals of all ages including children, especially those with underlying health conditions [5, 6]. Children account for 20-30% of the annual attack with those from developing countries recording 99% of deaths due to influenza related infections [4]. Over 250,000 African children, less than 5 years are hospitalized with influenza and have annual influenza associated hospitalization rate three times higher than the children in developed countries [7]. Unfortunately, in Nigeria, there is lack of a national policy on vaccination against this vaccine preventable disease either in the general population or among the high risk group.

Malaria on the other hand, is a parasitic disease caused by a single-celled protozoan of the genus *Plasmodium* belonging to the phylum *Apicomplexa* [8]. It is spread from person to person through the bite of infected female *Anopheles* mosquito [9]. Out of the 3.2 billion people at risk of malaria infection, about 214 million cases occur yearly particularly in Africa with Nigeria having the highest number of cases [10, 11]. It leads to 438,000 deaths with sub-Saharan Africa accounting for 90% [11], of which more than two-third occur in children less than 5 years old [12]. In Nigeria, malaria accounts for 60% of outpatient visits, 30% child mortality and 11% of maternal mortality [13]. Generally, children with malaria often present with fever, chills, headache, myalgia, and vomiting which is similar to some of the influenza symptoms [14].

Typhoid fever is a systemic infection caused by Gram negative bacterium *Salmonella enteric Typhi* (*S. Typhi*) which is transmitted from person to person through faecal-oral route [15]. Symptoms include fever, diarrhoea, loss of appetite and abdominal cramp [16]. Typhoid fever remains a significant health problem particularly among children and adolescent in developing countries [17] as a result of poor sanitation and unsafe food and water supply usually contaminated with human faeces [18]. In some areas, the incidence rate occurs among children below 5 years [19]. However, it was estimated in 2010 that typhoid fever caused 26.9 million cases with 217,000 deaths globally [20].

Children in sub-Saharan Africa including Nigeria are vulnerable to influenza, malaria and typhoid co-infection with little or no information on their coexistence. Therefore, this study was aimed to determine the seroprevalence of influenza, malaria and typhoid fever coinfection in children from different healthcare centres in Lagos, Nigeria.

Methods

Study site and subjects

A prospective cross-sectional sero-epidemiologic hospital-based study was designed. In and out-patients were recruited from government/ public health institutions in four different locations including Festac (Maternal and Child health), and three primary health centres (PHCs) of Amuwo, Ojo and Shibiri in Lagos State. The PHCs were chosen based on different local council development areas with close proximity to the University research laboratory in Ojo town, availability of screening facilities for routine malaria and typhoid tests, and the population of nursing mothers attending such centres for paediatric treatment. The maternal and Child centre is a reference paediatric centre for the treatment of infants, and children. It also provides pre-natal, postnatal, family planning and other medical services to mothers. Blood were collected from unvaccinated children between the months of March to October, 2018. The concept of the study was explained to their parent/ guardian and their consent sought. Information on demography, clinical symptoms, and other epidemiological parameters under confidential disclosure were appropriately collected with interviewer questionnaire printed-forms by trained Scientists and laboratory personnel.

Study population

In- and out-patients' from both sexes and age group 0– 14 years particularly those having influenza-like-illness (ILI) of fever above 38°C, runny nose/ catarrh and cough were recruited. Malaria and typhoid tests were routinely requested by the clinician for such children, while influenza was performed for the purpose of the study. The study excluded adults, patients without fever and those not tested for malaria and typhoid. The minimum sample size was determined using Kish, Leslie formula of $n = Z^2 p (1 - p) / d^2$, where Z= reliability coefficient of 1.96 at 95 % confidence interval. A presumed prevalence of 18.9% was used according to [21].

Ethical Approval and Permission

Ethical approval was obtained from the Health Research and Ethics Committee of Lagos State University Teaching Hospital (LASUTH) Research and Ethics Committee (LREC) with reference number: LREC.06/10/1030 while each hospital/ centre permission was sought from the head of the health centre/ hospital where the samples were collected.

Sample collection and preservation

Five (5) mls of blood samples were aseptically drawn by the phlebotomists or midwives using sterile needle and syringe. Each blood sample was shared into labeled EDTA and plain container for malaria/ typhoid and influenza viral assay respectively. Malaria and typhoid samples were further processed at the PHC of collection while sample aliquots for influenza viral assay were then kept in the coolers with ice-packs before being transported to the laboratory. Sera were separated from the blood by centrifugation at 3000 rpm for 10-15 minutes and transferred into labeled cryovial tubes with the aid of Pasteur pipettes, before they were stored at -30°C until ready to use.

Laboratory analyses

Laboratory analysis was performed at the Virology Research Laboratory, Department of Microbiology, Lagos State University, Ojo. All the children tested by Rapid Diagnostic Test (RDT) for the detection of malaria histidine-rich protein II (HRP-II) and *Plasmodium* lactate dehydrogenase (pLDH) (Accessbio or Medicon BioCheck, USA) and typhoid (CTKBiotech, USA or Omega, UK) (depending on the type of assay kit in use at the PHC), were further screened for the qualitative and quantitative determination of influenza virus specific IgM antibody by ELISA (Demeditec, Germany). Characteristics of the assay kit include: 100% clinical specificity and 100 % sensitivity with no cross-reactivity to RSV, Adenovirus and Parainfluenza 1/2/3 according to the manufacturer.

Briefly, for the malaria and typhoid RDTs performed at the PHCs, whole blood or plasma (as indicated in the manufacturer's assay procedure) was applied into the sample pad. Buffer solution (2 drops) were added (when whole blood sample was used) into the test kit round hole. Result was read and interpreted after about 10-15 minutes. Appearance of line in both the control and test region was recorded as positive result, otherwise, the result was negative or invalid. Invalid results were however repeated.

For the ELISA, micro titer wells were labeled for the standards, controls and samples as well as for substrate blank. The samples were diluted with ready to use sample diluents provided with the test kit in the ratio 1:101 (1 µl serum + 100 µl sample diluents). 100 µl of the ready to use standard controls and diluted samples were pipetted into the micro titer wells leaving one well empty for the substrate blank. The micro plate was covered with re-usable plate cover and incubated at room temperature for 60 minutes. Unused reaction fluid was emptied and 300 µl of diluted washing solution was added. The wells were washed three-times while remnant fluid was removed by gently tapping the micro titer plates on disposable papers. Ready to use enzyme conjugate (100 µl) was added into the wells leaving one well for the substrate blank. The microplates were covered with re-usable plate-cover and incubated at room temperature for 30 minutes. The plates were emptied and another 300 µl of diluted washing solution was added (three times) for washing. Remnant fluid was again removed by tapping the plate gently on disposable papers. Ready to use substrate (100 µl) was added into the wells and the substrate blank. The plate was covered and incubated at room temperature in the dark for 20 minutes. A blue colour was observed before the substrate reaction was terminated with 100 µl of ready to use stop solution. A yellow colour developed while the absorbance of the wells was read with ELISA microplate reader (Emax precision, Molecular Devices, California, USA) at 450 nm wavelength. Each assay batch was performed with positive and negative controls. All the test results were expressed based on the standard curve provided with the assay kit supplied by the Manufacturer. Samples were considered positive if the antibody concentration was >1.148 U/mL based on cut-off specification of the manufacturer. Positive influenza virus-specific IgM antibody has the implication of recent immunological response to infection with circulating influenza strains in the population as influenza vaccination is not practice in Lagos and Nigeria in general.

Statistical Analysis

Epidemiological data were systematically analysed using GraphPad Prism Version 8.0.1 (244), San Diego, USA. P-values and statistical significant differences ($p < 0.05$) between categorical data were measured using Chi-square.

Results

This prospective study investigated a total of 364 children having ILI from 4 health centres in Lagos (Fig. 1). They were previously screened for both routine malaria and typhoid fever tests while this study further examined those with both malaria and typhoid result outcome for possibility of co-infection with influenza virus. Out of the 364 blood samples investigated for IgM antibody specific to influenza virus, 20.9% (76/364) were seropositive. Of the 76 influenza seropositive children, 61.8% (47/76) were positive for malaria, 55.3% (42/76) were positive for typhoid, and 27.6% (21/76) had co-infection of both malaria parasitaemia and typhoid fever (Fig. 2).

Out of the 76 influenza seropositive patients, Ojo primary health centre had statistically significant highest seroprevalence of 48.7% (37/76) while the lowest 13.2% (10/76) seroprevalence was detected in

Festac town area of Lagos State. The highest seropositivity of 55.3% (42/76) was obtained in the age group 1-4 years while age 0-5 months recorded the lowest prevalence of 4% (3/76). The observable difference ($p = 0.0001$) was statistically significant. Of the 207 males examined, 61.8% (47/76) were seropositive to influenza virus compare to 38.2% (29/76) out of the 157 female children examined. The difference was however not statistically significant. In addition, 2/76 (2.6%) children having underlying health condition of sickle cell recorded influenza seropositivity (Table 1).

Figure 3 shows the clinical symptoms of seropositive children to influenza having co-infection of both malaria parasitaemia and typhoid fever. Fever 27.6% ($n=21$) was the most common of all the clinical symptoms recorded.

Although the month of March had the highest seroprevalence of 26.3% (20/76), seropositivity was recorded in all other months considered for this study (Fig. 4).

Discussion

Enzyme linked immunosorbent assay (ELISA) is a sensitive and quantifiable diagnostic method that has been used in the detection of different aetiologies [22]. Sera antibodies are often used to correlate influenza detection in the assay [23]. This study detected a total percentage seroprevalence of 20.9% to influenza virus among paediatric patients attending different healthcare centers in Lagos, Nigeria. Healthcare attendees were studied in order to increase the likelihood of detecting exposure of humans to the pathogens since parents of children with febrile illness or influenza-like illness are more likely to seek healthcare attention. The finding is similar to the prevalence of 22.1% influenza infection reported in Russian children between 2013 and 2017 [24]. A study conducted in Germany (2008- 2010) showed a higher IgG seroprevalence of 87.6% to influenza virus using ELISA technique [25]. The observable difference could be due to the measurement of IgG, an indication of past exposure to infection compared to the IgM ELISA used to measure recent infection in this study.

On the contrary, Dilantika, Sedyaningsih [26], Norowitz, Kohlhoff [27], and Alsuwaidi, Al-Mekaini [28] using ELISA method reported lower seroprevalence of 11.6% and 14.9%, and 15.8% respectively to influenza virus in children. In Africa, studies have reported influenza in pregnancy, HIV patients, sickle cell anaemia patients, and tuberculosis patients with prevalences of 48%, 3.9%, 2.0%, and 10% respectively [29-32] while others have shown co-infection of influenza with asthma, pneumonia and malaria but there is dearth of information on co-infection of influenza with typhoid fever, and malaria and typhoid, hence the importance of this study.

Our findings showed that a good number of children having monotypic seropositivity to influenza virus had co-infection with malaria parasitaemia and/or with typhoid fever with 61.8% co-infection of influenza and malaria higher than that of 55.3% for influenza and typhoid, and 27.6% co-infection of the trio of influenza, malaria and typhoid. Studies have shown that influenza is one of the viruses that may lead to complication of malaria infection [33, 34], while findings from this study is suggestive of malaria parasitaemia complicating influenza infection in children. However, Thompson, Breiman [35] reported

uncommon co-infection of malaria and influenza in Kenya in 2009-2011. A good reason for this might be that both malaria parasitaemia and influenza can be sub-clinical or incidental to the presenting illness [36, 37].

Our findings of 55.3% monotypic infection of influenza, 31.9% co-infection of influenza and malaria, 50% coinfection of influenza and typhoid, and 42.9 % coinfection of influenza, malaria and typhoid respectively showed that children in the age group 1-4 years were the most affected by all the infections when compared to other age groups and the difference was statistically significant (Table 1). This is a pointer that children of age 1- 4 years had the highest recent exposure to influenza virus, mosquito bite and contamination with *S. typhi*, that call for urgent and serious public health attention. The influenza monotypic infection unlike influenza, malaria and typhoid co-infection is consistent with the study of [Tempia, Walaza \[38\]](#) that reported a similar prevalence of influenza virus in children less than 5 years of age. Studies from India and Bangladesh also reported highest prevalence of influenza in children less than 5 years [39, 40]. [Gessner, Shindo \[36\]](#) showed that in Sub-Saharan Africa, children less than 5 years are more exposed to influenza than other age group. According to [CDC \[41\]](#), the highest hospitalization for influenza epidemiology was reported in children age 0-4 years with the lowest prevalence in children of 5-14 years. Our finding is also consistent with that of [Riquelme, Torres \[42\]](#) that estimated high rate of influenza infection in children less than 5 years, unlike [Mancinelli, Onori \[43\]](#); and [Shang, Blanton \[44\]](#) that concluded that influenza seropositivity was highest among children less than 1 year. This could be argued with the fact that their immune system is immunologically immature, making them to be more susceptible to influenza [45].

The male children had higher 61.8% (47/76) IgM seroprevalence to influenza virus unlike their female counterpart with 38.2% (29/76). This is similar to the finding reported by [Tivane, Daniels \[31\]](#) that influenza virus is higher among male children. This position was also supported by [Mancinelli, Onori \[43\]](#).

The commonest 27.6% (n= 21) clinical symptom of influenza virus presented in the study was fever. This agrees with the finding of [Taşar, Bilge \[46\]](#) that fever is the commonest symptom of influenza-like-illness.

Exposure to recent influenza activity was confirmed through-out the months in which samples were collected with a higher occurrence recorded in the month of March. This is in line with the findings of [Tivane, Daniels \[31\]](#) which showed that influenza occurred through-out the year but the activity was peak in February and August. In contrast to this study, [Sanou, Wandaogo \[47\]](#) showed that influenza was most prevalent in September to October. The basic difference in the prevalence at each point in time is due to the favourable weather condition.

Children having underlying health condition of asthma in this study tested negative to IgM seroprevalence to influenza virus. This finding agrees with the earlier investigation by [Tivane, Daniels \[31\]](#) which opined that no relationship exist between children having asthma and influenza. However, previous studies have shown correlation between influenza, sickle cell and asthma [48] in support of the 2.6 % seroprevalence of influenza in sickle cell patients detected in this study. This is further supported by [Bundy, Strouse \[30\]](#) that reported prevalence of influenza in children with sickle cell. According to

Adewoyin [49], Nigeria has 20%– 30% carrier rate for sickle cell gene with sickle cell disease affecting 2%– 3% of the entire population. However, influenza with malaria parasitaemia and typhoid fever coinfection must be urgently prevented in such individuals in order to prevent likely fatal consequences.

Part of the limitations of this study is that we were unable to further analyse the ELISA positive samples by molecular assay due to limited resources. Therefore, there is need for a future study that can generate nucleotide sequences for global data sharing and possible consideration as vaccine candidates from sub-Saharan Africa.

Conclusions

Our investigation revealed specific IgM antibody seroprevalence to influenza virus with 27.6% co-infection of malaria parasitaemia and typhoid fever among children of all age group round the months. Fever represented the most common clinical symptom while low co-infection was recorded in sickle cell anaemia patients. Differential screening is hereby recommended for accurate and effective treatment while annual vaccination is strongly advocated for all children in order to prevent coinfection of influenza with other deadly diseases.

Declarations

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Availability of data and materials

The datasets used and analysed during the study are included within the article. Additional data can be provided from the correspondence upon reasonable request.

Authors' contributions

Conception and Design: AAA. Execution and Interpretation: AAA, ABS, IOP. Manuscript writing: AAA, ABS. All authors read and approved the manuscript.

Ethics approval and consent to participate

The study procedure was approved by the Health Research and Ethics Committee of Lagos State University Teaching Hospital (LASUTH) Research and Ethics Committee (LREC) with reference number: LREC.06/10/1030. Written informed consent was obtained from the parent/ guardian of all the participants.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1: Epidemiological Data of Children co-infected with Influenza, Malaria and Typhoid fever in Lagos, Nigeria

Epidemiological Data	Mal (%) (n=248)	Typ (%) (n=146)	Positive for Flu (%) (n=76)	Flu+Mal (%) (n=47)	Flu+Typ (%) (n=42)	Flu+Mal+Typ (%) (n=21) <i>P value</i>
Location						
<i>Ojo</i>	171	100	37 (48.7)	29 (61.7)	22 (52.4)	13 (61.9) 0.04
<i>Shibiri</i>	(69)	(68.5)	15 (19.7)	11 (23.4)	8 (19)	7 (33.3)
<i>Amuwo</i>	30	20	14 (18.4)	4 (8.5)	7 (16.7)	1 (4.8)
<i>Festac</i>	(12.1)	(13.7)	10 (13.2)	3 (6.4)	5 (11.9)	0 (0)
	27	12 (8.2)				
	(10.9)	14 (9.6)				
	20 (8)					
Gender						
<i>Male</i>	136	85	47 (61.8)	29 (61.7)	30 (71.4)	13 (61.9) 0.43
<i>Female</i>	(54.8)	(58.2)	29 (38.2)	18 (38.3)	12 (28.6)	8 (38.1)
	112	61				
	(45.2)	(41.8)				
Age Range						
<i>0-5 months</i>	17 (6.9)	10 (6.8)	3 (4)	14 (29.8)	6 (14.3)	5 (23.8) 0.0001
<i>6-11 months</i>	24 (9.7)	16 (11)	7 (9.2)	10 (21.3)	10 (23.8)	3 (14.3)
<i>1-4 yrs</i>	130	40	42 (55.3)	15 (31.9)	21 (50)	9 (42.9)
<i>5-9 yrs</i>	(52.4)	(27.4)	15 (19.7)	5 (10.6)	4 (9.5)	4 (19)
<i>12-14 yrs</i>	46	51	9 (11.8)	3 (6.4)	1 (2.4)	0 (0)
	(18.5)	(34.9)				
	31	29				
	(12.5)	(19.9)				
Underlying Health Condition						
<i>Sickle cell</i>	10 (4)	6 (4.1)	2 (2.6)	1 (2.1)	0 (0)	0 (0)
<i>Asthma</i>	0 (0)	2 (1.4)	0 (0)	0 (0)	0 (0)	0 (0)

Legend: Flu, influenza; Mal, malaria; Typ, typhoid.

Figures

Figure 1

Flow Diagram for inclusion and sampling of children with influenza-like illness (ILI) screened for malaria (M) and typhoid (F).

Figure 2

Distribution of Influenza coinfection with Malaria parasitaemia and Typhoid fever among Healthcare attendee Children in Lagos, Nigeria. Legend: Flu, influenza; Mal, malaria; Typ, typhoid.

Figure 3

Distribution of clinical symptoms among children having coinfection of influenza, malaria parasitaemia and typhoid fever in Lagos, Nigeria.

Figure 4

Distribution of Influenza, Malaria parasitaemia and Typhoid fever coinfection in Children from March to October in Lagos, Nigeria.