A preliminary Study on Neutralizing Antibody to Poliovirus in Adults in Maiduguri, Nigeria.

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Abstract: Poliomyelitis is one of the debilitating diseases due to its ability to cripple and cause death in adults, children and adolescents. With the aim to assess the current immunity profile of adults in this environment as epidemics have strucked in some countries which resulted in great mortality and morbidity. Of the serum samples of 200 subjects studied by microneutralization technique, only 181(90.5%), 160(81%) and 171(85.5%) had antibody titres for P1, P2 and P3 respectively. Only 25% of adults studied had neutralizing antibodies to the three serotypes of polioviruses. 19(9.5%), 38(19.0%) and 29(14.5%) had no neutralizing antibody for P1, P2 and P3 respectively. A significant number of the adults studied had no detectable neutralizing antibodies to the three serotypes of polioviruses. The ages of the seropositive subjects ranged between ≤19 and ≥ 70 years with the highest titre at the age range of 20-29. Most of the study population had antibody to either a single or combination of two poliovirus serotypes indicating partial immunity and this poses the risk of infection with the wild strain of the missing serotype. Therefore, lack or partial immunity to poliovirus observed in this study may impede the success of polio eradication program in Nigeria.

Key words: Neutralizing antibodies; Poliovirus; Seropositive; Immunity; Adults; Maiduguri

INTRODUCTION

As the world envisions poliomyelitis eradication, objective parameters are needed to ascertain whether immune competence against the virus, provided by vaccine campaigns, has been attained in key areas. The presence of neutralizing antibodies is considered a surrogate marker of protective immune response to the agent (Spaendonck Conyn-Van, et al., 2001). Although immunosurveillance is not required by the World Health Organization, but it will provide in sight into the protective immunity of sub populations. Immunosurveillance will be of added value in eradicating polio, particularly in countries with pockets of unvaccinated persons (Van Loon, A.M., et al., 1998; Mensi, C. and Pregliasco F., 1998; De Melker, H.E. and Spaendonck Conyn-Van, M.A.E., 1998). In Japan that is a PV-free (Poliovirus-free) country, surveillance of neutralizing antibody against PV(Poliovirus) has been performed every 2 or 3 years since 1974 from serum samples of healthy volunteers (about 1,100 to 1,800 individuals in 6 to 8 prefectures) in a wide range of ages (0 to > 40) in prefectural laboratories (http://idsc.nih.go.jp/yosoku/Polio/Year-P2009.html) (Iwai, M., et al., 2008).

According to Atkinson (2011), the death-to-case ratio for paralytic polio is generally 2% - 5% among children and up to 15% - 30% for adults depending on age and increases to 25% - 75% with bulbar involvement which shows that the mortality and morbidity rate is higher in adults than in children even in past epidemics records.

In the end game of polio eradication program, serosurveillance for antibodies against poliovirus in susceptible populations is essential for monitoring vulnerability to poliovirus circulation in PV-free countries to sustain their PV-free status and the seroconversion rates in countries where PV is endemic to evaluate the effectiveness of vaccination strategies (Arita, M., et al., 2011). The aim of this study was to investigate the level of immunity in adults against poliovirus serotypes 1, 2, and 3 in Maiduguri, Nigeria.

MATERIALS AND METHODS

Study area:
Serum samples were collected from adults who visited University of Maiduguri Teaching Hospital (UMTH), Maiduguri, Nigeria for medical attention and voluntary blood donation. The hospital is a tertiary health institution located in Borno State, Nigeria and serves as a referral health centre for six states (Adamawa, Bauchi, Borno, Gombe Taraba and Yobe,) in northeastern Nigeria and neighboring African countries (Chad to the northeast, Niger to the north and Cameroon to the east). Based on the 2006 provisional census figures, Borno

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State has a population of 4,151,193 and a population density of approximately 60 inhabitants per square kilometer. The state has an area of 61,435 sq.km (the largest state in Nigeria in terms of land mass). (www.bornonigeria.com/index.php).

**Study Population:**

The Ethical Committee of University of Maiduguri Teaching Hospital, Maiduguri gave approval to the study protocol. Only apparently healthy blood donors and adults with no known immune disorder or who had been treated with immune-suppressive drugs or radiotherapy in the previous twelve months or had received systemic corticoids in the last three months or had received immunoglobulin or blood transfusion in the last three months were excluded from the study. After obtaining informed consent from those subjects, willing participants were enrolled consecutively in the study. Demographic data were collected.

**Vaccination History Of The Subjects Studied:**

The record of vaccination history of the subjects tested was not available but information in this regard was obtained was verbal. However, it may be necessary to state that none of the subjects could confidently say he has been vaccinated for polio.

**Sample Collection:**

A total of 200 serum samples were collected from the adults studied between January and February 2012. About 2 ml of blood was collected, allowed to clot, centrifuged and the serum was aspirated into the sterile cryovial. The serum samples were transported within 24 hours to the World Health Organization National Polio Laboratory, Maiduguri for storage and analysis. All the sera were stored at -20°C until tested.

**Microneutralization Assay:**

Neutralizing titres against poliovirus were carried out according to WHO guidelines (WHO Polio Laboratory Manual, 2004). Sera were inactivated at 56°C for 30 minutes. Sera were serially diluted in two-fold from 2 to 2048, and 50ul of sera with 50ul of 100 tissue culture infective dose 50 (TCID50) of challenge virus in triplicate (PV1, 2 and 3) National Institute of Biological Standards and Controls Reference strains (Sabin 1; 01/528, Sabin 2; 01/530, Sabin 3; 01/532) from which calibrated in –house reference strains were produced as described in WHO Polio Manual (2004) with known titre in cell sensitivity on L20B. Then each dilution was incubated for one hour at 36°C to allow the antibodies bind to the virus present. Subsequently, suspensions of 2 x 10⁵ cells/ml of L20B cells were added to the virus-serum mixtures in the micro wells. Cell controls and a local in-house reference serum of known PV1, 2 and 3 neutralizing titre was included in each test for standardization and reproducibility of results. After 5 day incubation at 36°C, the highest dilution of serum that prevents the development of virus induced cytopathic effects (CPE) was recorded. The NA titre corresponded to the reciprocal of this dilution. A titre of ≥1:8 were defined as indicative of protected immunity.

**Statistical Analysis:**

The results of neutralization test analyzed using Epil Info 6 software (Centers for Disease Control and Prevention). Age, sex, and the prevalence of neutralizing antibodies of the studied population were compared. χ² was used for statistical testing and the level of statistical significance chosen for all analyses was p < 0.05.

**Results:**

A titre of ≥ 1:8 was defined as indicative of protected immunity (Luchs A., *et al*., 2010; Pires de Miranda, M., *et al*., 2007). The total sample size for this study was 200. 40(20%) were male and 160(80%) were female.

<table>
<thead>
<tr>
<th>Description</th>
<th>Number tested (200)</th>
<th>Percentage positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positives for P1</td>
<td>139</td>
<td>69.5</td>
</tr>
<tr>
<td>Positives for P2</td>
<td>98</td>
<td>49</td>
</tr>
<tr>
<td>Positives for P3</td>
<td>107</td>
<td>53.5</td>
</tr>
<tr>
<td>Positives for P1, P2, and P3</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td>Positives for P1 and P2</td>
<td>83</td>
<td>41</td>
</tr>
<tr>
<td>Positives for P1 and P3</td>
<td>78</td>
<td>39</td>
</tr>
<tr>
<td>Positives for P2 and P3</td>
<td>62</td>
<td>31</td>
</tr>
</tbody>
</table>

Out of 200 adults studied 139(69.5%), 98(49%) and 107(53.5%) were positive for P1, P2 and P3 respectively. 50(25%) were seropositive for the three serotypes.
Out of 200 adults studied, only 34(17%) males and 102(51%) females were seropositive for P1. Seropositive individuals for P2 were 76(38%) females and 22(11%) males. Seropositive males for P3 numbered 18(9%) and females numbered 89(44.5%). For the three serotypes, P1, P2 and P3, 18(9%) males and 36(18%) of females were seropositive. Overall, the sex of the patients were significantly different from the neutralizing antibodies to the different serotypes of polioviruses.

Seropositive individuals between age ranges of 20 – 69 years had high values for neutralizing antibodies to serotype P1 compared to P2 and P3. Those 20-29 years had the highest values for neutralizing antibodies to P3 compared to other age ranges. The older population (≥ 70), had neutralizing antibodies to only P1 and P2, and none for P3. Those ≤ 19 years had the lowest number of seropositive individuals for P1.

Relationship Between Age, Gender And Neutralizing Antibodies:

The prevalence of neutralizing antibodies and the sex of the adult population studied was significantly different (≤ 0.05). However, the highest titre of 1:1024 against P3 was obtained in a male but lower titre in females.
In this study, the neutralizing antibodies to polioviruses were highest among those aged 20-29 years for P1 and P2 compared to P3. No significant difference (≥ 0.05) between the ages of the adults and the antibody prevalence was observed.

**Discussion:**

Public health surveys carried out in line with the polio eradication campaign have recorded a steady rise in polio cases among adults in Africa. Polio previously referred to as a childhood disease, affected persons aged 1-72 years in pointe –Notre, Congo in September 2010 to February 2011 (www.cdc.gov/eid, 2011). The majority of those affected were young adults between the ages of 15-24 years. A total of 184 deaths were reported, and similar death rates have been reported in previous outbreaks in Cape Verde (CDC, 2000), Albania (Prevots, D.R., et al., 1998) and Namibia (www.afro.who.int/en/media-centre/pressrelease/it.). This occurred because most children (who are today adults) failed to be vaccinated and this eventually led to low immunity in the adult community.

Out of 200 adults studied 139(69.5%), 98(49%) and 107(53.5%) were positive for P1, P2 and P3 respectively. 50(25%) were seropositive for the three serotypes. The findings of this study implies that 75% of adults residents in Maiduguri, Borno State do not have neutralizing antibodies to the three serotypes and are at the risk contracting the wild polioviruses. The partial immunity to either a single serotype or combination of two still exposes such individual to infection with the missing serotype (s). This speculation is supported by a report that, Borno is one of the three northern States accounting for 68% of polio cases in Nigeria (www.polioeradication.org.Polio, 2012). It may be necessary to state that outbreaks of poliomyelitis among adults have been reported in countries such as Congo Republic, Cape Verde, Albania and Namibia (www.afro.who.int/en/media-centre/pressrelease/it. 2011.), due to lack of neutralizing antibodies to polioviruses. In Point-Notre, Congo Republic, 184 deaths were reported while previously, 6 deaths occurred within May to June in 2006 in Namibia. 17 deaths were reported in 2000 in Cape Verde (www.globalhealthfacts.org, 2001), while in 1996, 16 deaths resulting from outbreaks occurring in April to December were recorded in Albania (CDC, 1998).

Generally, the highest population (69.5%) studied had neutralizing antibody to P1 compared to P3 (53.5% and P2 (49%) This observation may be associated with intensive immunization exercises to children 0-5 years with monovalent polio vaccine 1 and 3 because wild poliovirus (WP2) had been eradicated since 1999 (CDC, 2001).The prevalence of antibody to either a single or combination of two serotypes obtained in this study is in consonance with previous report in the same environment (Baba, M.M., et al., 2012). However, while the previous report used children 0-5 years, this study was restricted to adults from 18 to 80 years. The similarity in the pattern of antibody profile among both adults (in this study) and children (Baba, M.M., et al., 2012) seems to reflect the nature of vaccine used during vaccination exercises. It could then be speculated that, the adults probably got immunized through the vaccine strain from vaccinated children because National Program on Immunization are targets children under five years and not adults. Therefore, there is need for further studies to ascertain the immune status of the adult in other communities, not only in Maiduguri but in Nigeria at large in a bid to avert any pending outbreak.

The prevalence of neutralizing antibodies and the sex of the adult population studied was significantly different (≤ 0.05) in contrast to previous report in the same environment although children 0-5 years were used in the previous study (Baba, M.M., et al., 2012). However, the highest titre of 1:1024 against P3 was obtained in a male but lower titre in females.

In this study, the neutralizing antibodies to polioviruses were highest among those aged 20-29 years for P1 and P2 compared to P3. No significant difference (≥ 0.05) between the ages of the adults and the antibody prevalence was observed in contrast to a previous study (Adewumi, M.O., et al., 2006) among children in the South western Nigeria. However, adults aged 70 years and above had no protective antibody to P3 and are at the risk contracting the wild poliovirus. In conclusion a significant number of the adults studied had no detectable neutralizing antibodies to the three serotypes of polioviruses. Also, a significant number of these adults had antibody to either a single or combination of two poliovirus serotypes. Therefore, lack or partial immunity to poliovirus observed in this study poses a serious threat to the success of polio eradication program in north eastern Nigeria. There may be need to include adults in polio eradication program to avoid outbreaks as it occurred in other countries. Further study on antibody profile to polioviruses in adults at a wider scale would support or reject the idea of adult inclusion in polio immunization in Nigeria.

In recommendation, in several African countries the vaccination coverage against poliomyelitis has not reached optimum levels, although governments and humanitarian organizations have made numerous efforts in organizational and monetary terms. Wars and especially religious beliefs, have presented obstacles to a thorough diffusion of polio vaccination. In the light of this, periodic assessment of immunity levels in the population and particularly in the more vulnerable sub-populations, like immigrants and refugees, is necessary. This must be done together with environmental monitoring of viral circulation and surveillance of acute flaccid paralysis (Aylward, R., et al., 2006). Identifying the missed children as well as adults, and vaccinating them should form
part of the focus of the eradication efforts. Sustenance of intense OPV administration is another key point. Full implementation of the surveillance action plan, with particular attention to enhancing active surveillance in major hospitals can help solve this immunity gap. Elimination of non-compliance to polio vaccine, promotion of health education and documented evidence of vaccination of each child with the parents may facilitate the success of polio eradication program in Nigeria.

ACKNOWLEDGEMENTS

We thank the WHO for provision of technical support to the laboratory and management of UMTH for their contribution to the study through patients and blood donors’ enrolment.

REFERENCES