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Research Paper

IMMUNOTOXICOLOGICAL RESPONSE OF SCHOOL AGE CHILDREN TO ENVIRONMENTAL POLLUTION AND HETEROPHILE ANTIGENS IN RIVERS STATE, SOUTHERN NIGERIA

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Abstract

Natural and anthropogenic activities threaten our health and ecosystem. Thus, everyday exposure to environmental pollutants even at smaller doses may interfere with key immunological processes resulting in high-risk immune-enhancement or immunosuppression with increased disease vulnerability. The study assessed the prevalence of heterophile antibodies and immunoglobulin concentrations in 96 urban and rural children in Rivers state, Nigeria, in response to unknown environmental pollutants. Subjects were obtained from Port-Harcourt, Gokana and Emuoha LGAs; all ethical considerations were satisfactorily met. Antigenic challenge was induced by exposing human sera to sheep red blood cells-(SRBCs) using double-dilution technique. Serum IgA, IgG, IgM were determined using radial diffusion method. Results showed significant ($p<0.05$) heterophile agglutinins reacting with SRBCs at 1:2 titre or greater; high incidence-(96.9%) of heterophile antibodies proportionate to age of subjects. On incubation, some human serum precipitated. IgA, IgG, IgM were significantly higher in urban children's sera. The increased immunoglobulin suggests routine exposure to environmental toxicants; observed serum precipitation signifies presence of excess pyroglobulins, possibly indicating multiple myeloma or macroglobulinemia. Since exposure to immunosuppressive xenobiotic first manifests a form of immune-hyperactivity, a comparative immunological profile and its relationship with specific environmental

toxicants is recommended for regulatory/preventive medicine policy formulations.
Key words: Heterophile antigens, immunotoxicology, immunoglobulins, toxicant, environmental exposure.

INTRODUCTION

The immune system is a special system of biological structures and processes within an organism which primarily defends it from a range of invading pathogens, tumour cells, viruses, bacteria and parasitic worms. Such defence is more appropriately done by distinguishing the invading agents from the organism's own healthy cells and tissues, and is necessary for the proper physiology of the body [1-2]. For many clinical purposes, the immunological profile of humans are often evaluated and measured. This occurs especially in children, since they are prone to a high prevalence of routine exposure to disease-causing agent both in the urban and rural setting.

Rivers state is one of Nigeria's oil producing states with a total area of 11,077 km^2, making it the 26th largest state in the country. Natural and anthropogenic activities (e.g. oil exploration, mining etc) of man present recurrent threats to human and environmental health. Thus, our everyday exposure to environmental pollutants even at small doses may interfere with key immunological processes resulting in either high-risk immune enhancement or immunosuppression with increased disease vulnerability. Though several researches have been done on adults' serum immunological profile, however, there is paucity of such data among school age children in Rivers state. Thus, the present study was designed to assay the prevalence of heterophile antibodies as well as immunoglobulin concentrations in the sera of 96 school age children of both sexes (5-12 years) drawn from urban and rural centres in Rivers state, Nigeria in response to unknown environmental pollutants as well as to determine the rate of children's vulnerability to infectious diseases judged based on their immune response following an antigenic challenge.

MATERIALS AND METHODS

Research Population and Study Location

A total of ninety six (96) children (60 males and 36 females) between the ages of 5 – 12 years were randomly recruited into the study, comprising of 48 rural and 48 urban children resident in Rivers state, Nigeria. The subjects were randomly drawn from Port-

Harcourt, Gokana and Emuoha local government areas of Rivers State, Nigeria [Figure 1].

Ethical Consideration

In line with the Helsinki declaration on researches on human subjects, the research was designed such that those who participated as subjects did so voluntarily. It was approved by the Ethics Committee, College of Health Sciences, University of Port Harcourt. The principle of beneficence and nonmaleficence was employed and the identity of subjects of from which blood samples were collected was kept confidential. Request-for-consent letters were introduced to the subjects, wherein the purpose, methods and benefits of the research were clearly stated. Parents/Guardians gave consent for blood samples to be collected from their children/wards in line with the Nuffield Council's recommendation for ethics [3].

Figure 1: Map of Rivers state showing the study locations. Source:http://www.researchgate.net/publication/258398776_ethnobotanical_studies_of_port_ harcourt_metropolis_Nigeria/figures?lo=1

Preparation of Alsever's Solution

The solution is a preservative (anti-coagulant) used to preserve sheep's red blood cells. This was prepared by diluting 20.5 g dextrose; 8 g sodium citrate, 0.552 g citric acid, and 4.2 g sodium chloride in a little amount of distilled water and made up to 1000 mL. Then the pH of the solution was adjusted to 6.1. The solution was then divided into aliquots of 25 mL, 25 mL, and 150 mL and dispensed into 3 conical flasks respectively. The solutions were autoclaved for 15 min at a pressure of 10 Lb for sterilization and stored at a temperature of 4 °C.

Collection of Sheep Red Blood Cells

After shaving off the desired area of puncture, 25 mL of the sheep' blood was collected from the tibial vein into each of the conical flask containing 25 mL aliquots of the Alsever's solution (already autoclaved) to make up 50 mL of solution. The two were then mixed to homogeneity and stored at 4 °C for 72 h to age.

Experimental Design

Blood sample was collected by venepuncture of the antecubital vein of the fore arm of children (5-12 years). As earlier described by Fahley *et al*. [4], antigenic challenge was induced by exposing the human sera to sheep red blood cells (SRBCs) using the double-dilution technique. Serum IgA, IgG and IgM were determined using radial diffusion method as earlier described by Mancini *et al.* [5].

Assay of Heterophile Antibodies

SRBCs were preserved in Alsever's solution (anticoagulant). This was spun at 3000 rpm for 5 min to separate the SRBCs from the anticoagulant. SRBCs was washed in normal saline and spun 3 times to have a clear and odour free supernatant. Using a smooth-edged Pasteur pipette, each well of the haemagglutination tray [Figure 2] was filled with 2 drops of normal saline (including the control wells). To the 9th well (positive control), 2 drops of the test sample (human serum) was added after which 2 drops was then added to the first well and mixed thoroughly. This was done carefully to minimize bubbles. 1/256 dilutions were made via the transfer of 2 drops of the mixture in the 1st well to the 2nd well and from the 2nd well to the 3rd well….up to the 8th well (1/256). To give equal volume of diluted serum, after thorough mixing in the 8th and 9th well, 2

drops were pipette and thrown off. This procedure was repeated for all the numbers of trays available. To each well (containing doubling dilutions of the test samples), 2 drops of 1% SRBCs was added, including the control wells. The samples were mixed to homogeneity by rocking, covered with a cling film to prevent evaporation and stored overnight at 4 °C. The wells with agglutination showed the titre values for the antigen-antibody reaction. Then the highest dilution that gave a positive reaction to each test sample was noted, read and recorded as a reciprocal of the highest dilution.

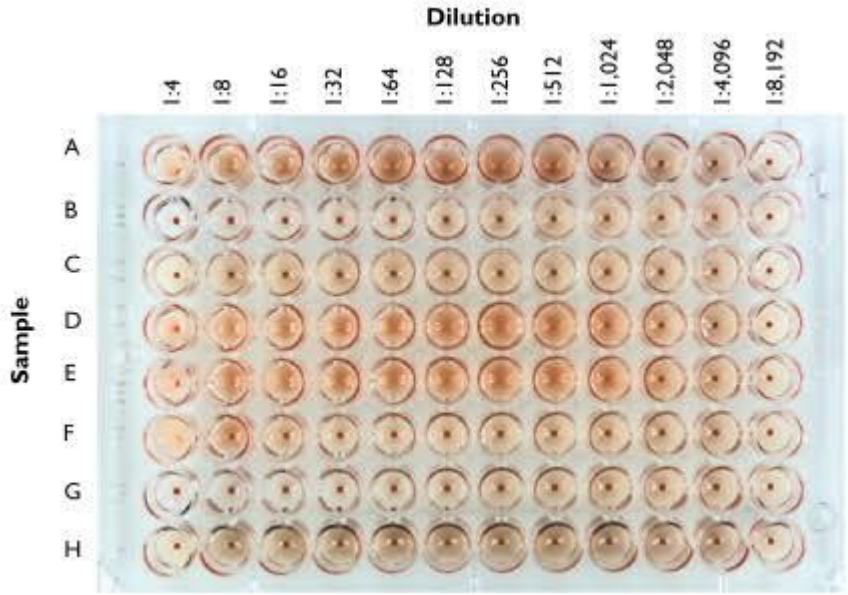

Figure 2: Haemagglutination tray showing double dilutions of test samples

Determination of Immunoglobulin (A, G & M)

This was done using plates made up of a mixture of noble agar and an appropriate dilution of human antisera (1.5 mL dissolved in 5.5 mL PO_4 buffer saline). The agar plates were stored in a humid box at 4 ºC for about 4 h. Thereafter, wells were made on the plates, agar carefully removed from the wells using suction pump and filled with suitable standards in duplicate of 200, 100, 50 and 25%; followed by the test samples using a micro-pipette [5]. The agar plates were reintroduced into the humid box at 4 °C for about 4 and 18 h for IgG, IgA and IgM respectively. The ring diameters formed on the wells were read to the nearest 0.1 mm with a metre rule and an oblique light source. The concentrations of the immunoglobulin in the test samples were obtained by the reading the concentration of the standard indicated by the ring diameter of the test

solution. The concentration of the standard against the ring diameters was plotted on a semi-log graph [Figure 4].

Statistics

Using SPSS software (version 16), the results were statistically analysed by one-way of variance (ANOVA) followed by Newman–Keuls as post hoc test and values less than ($p<0.05$) were considered significant.

RESULTS

Heterophile Antibody Titres

Results showed significant ($p<0.05$) heterophile agglutinins reacting with sheep RBCs at a titre of 1:2 dilutions or greater [Figure 3] as well as a high incidence (96.9%) of heterophile antibodies proportionate to the age of the subjects (Table 1). The subjects drawn from the rural centres were significantly older than those from the urban centres (Table 1). There was no significant difference in the heterophilic antibody titre of the rural and urban males. Rural females had higher heterophile antibody titres compared to their urban counterpart (Table 2).

Table 1. Heterophile antibody titres for urban and rural population

Parameters	Urban (n=48)	Rural (n=48)
Age (years)	6.75±2.18	9.40±1.74[a]
Hetrophile antibodies titre	65.84±100.62[a]	44.92±78.85

Data is expressed as mean ± SD, Significant at [a]*P* <0.05, (n = 96).

Table 2. Heterophile antibody titres for urban and rural females

Parameters	Urban (n=23)	Rural (n=13)
Age (years)	6.43 ± 2.15	9.15 ± 1.73[a]
Heterophile antibodies titre	21.74 ± 19.44	56.92 ± 77.14[a]

Data is expressed as mean ± SD, Significant at [a] *P* <0.05, (n = 36).

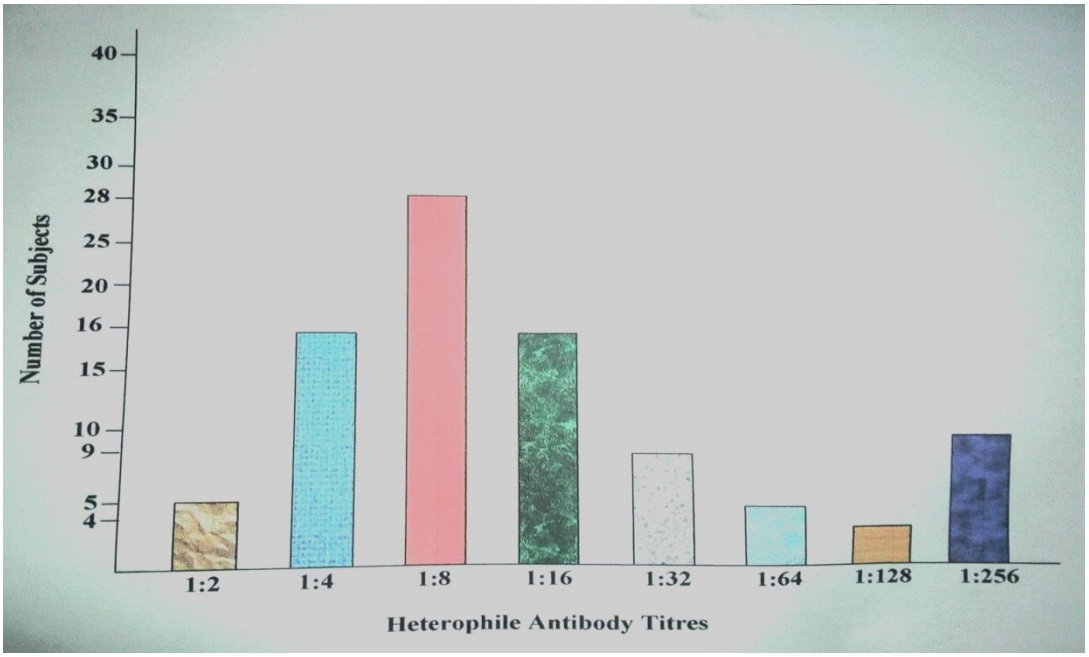

Figure 3. Heterophile antibody titres of research participants.

3.2. *Immunoglobulin Concentrations*

The results revealed that IgA, IgG and IgM were significantly higher in the sera of children drawn from the urban centres at values higher than the reference values (Figure 4; Tables 3 – 5).

Table 3. Immunoglobulin concentrations for urban and rural population

Parameters	Urban (n=48)	Rural (n=48)	Reference values
Age	6.75 ± 2.18	9.40 ± 1.74[a]	
IgA (mg/dL)	197.77 ± 147.79[a]	109.22 ± 62.75	188
IgG (mg/dL)	989.45 ± 296.35[a]	761.57 ± 29.87	934
IgM (mg/dL)	85.50 ± 14.97[a]	40.17 ± 15.62	69

Data is expressed as mean ± SD, Significant at [a] P <0.05, (n = 96).

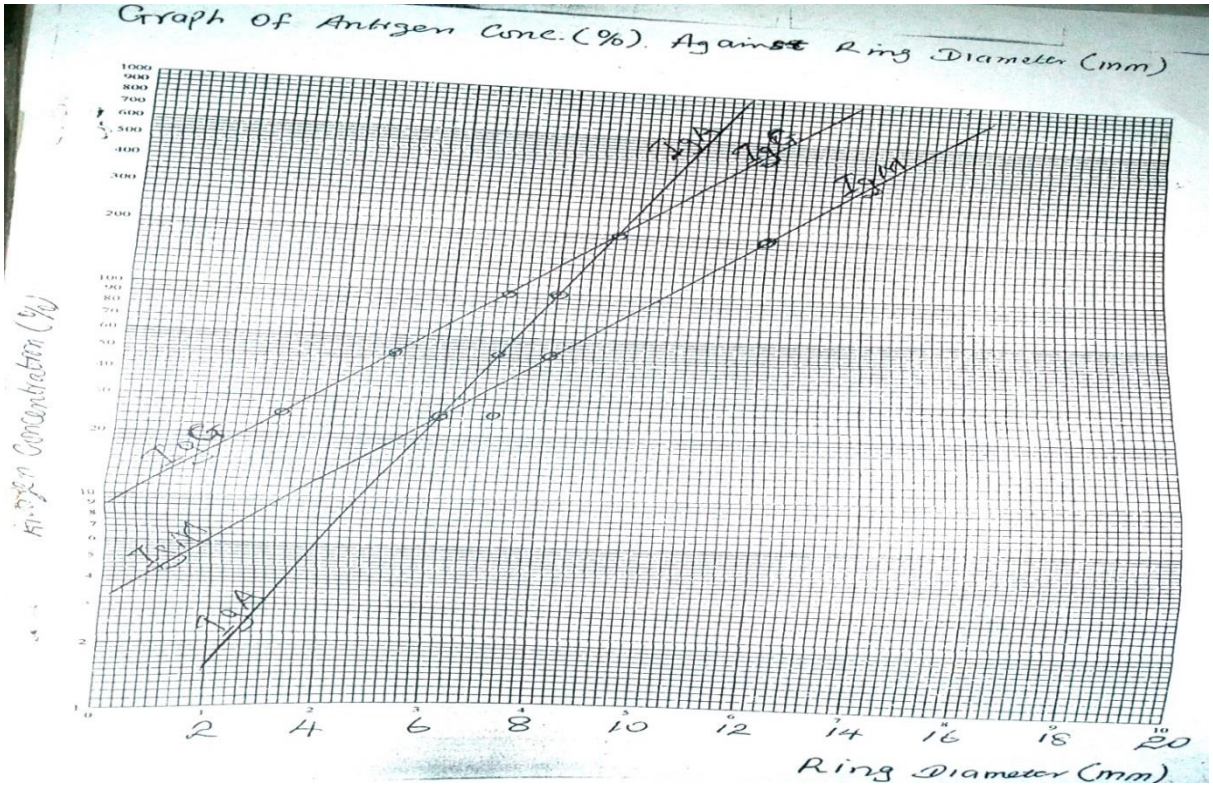

Figure 4: Graph of antigen concentration (%) against ring diameter (mm)

Table 4. Immunoglobulin concentrations for urban and rural males

Parameters	Urban (n=25)	Rural (n=35)	Reference values
Age	7.04 ± 2.21	9.49 ± 1.76[a]	
IgA (mg/dL)	200.38 ± 292.73[a]	163.48 ± 166.89	188
IgG (mg/dL)	950.17 ± 451.26[a]	621.92 ± 443.21	934
IgM (mg/dL)	83.74 ± 11.93[a]	43.70 ± 14.55	69

Data is expressed as mean ± SD, Significant at [a] P <0.05, (n = 60).

Table 5. Immunoglobulin concentrations for urban and rural females

Parameters	Urban (n=23)	Rural (n=13)	Reference values
Age	6.43 ± 2.15	9.15 ± 1.73[a]	
IgA (mg/dL)	137.77 ± 147.79[a]	89.22 ± 32.75	188
IgG (mg/dL)	897.45 ± 296.35	761.57 ± 299.87	934
IgM (mg/dL)	55.50 ± 14.97	48.17 ± 15.62	69

Data is expressed as mean ± SD, Significant at [a] P <0.05, (n = 36).

DISCUSSION

The observed significantly high titre values for heterophile antibody in rural females may be due to improper medical care and recurrent exposures of the rural females to infections. Since improved medical care since drugs e.g. NSAIDs, opioids etc. tend to suppress the rate at which the body produces its own natural antibodies [6]. The relatively high heterophilic antibody titres in urban subjects may be due to the prevalence of environmental toxins in the urban centres than in the rural environs [7, 8]. Higher concentrations of immunoglobulins recorded for the urban subjects suggest a routine exposure to environmental toxicants and is agreeable with the report of Gonzalez *et al.* [9], especially as exposure to immunosuppressive xenobiotic may first be manifested by a form of immune hyperactivity. On incubation at 56 ^{o}C, some of the human serum precipitated signifying the presence of excess pyroglobulins, indicating a probable multiple myeloma or macroglobulinemia [10].

In childhood, the maturation of immune responses is a continuous process [11]. For example, the adult level of IgG is reached by the seventh year of life after birth and remains relatively constant thereafter [12]. Meanwhile, the synthesis of IgM increases rapidly soon after birth and the adult level may be reached by the ninth month of age [13]. Haworth *et al*. [14] reported that the synthesis of IgA begins during the first few weeks after birth and that the concentration rises slowly during the first year and is continued throughout early adulthood. For many clinical purposes, the immunological profile of humans are often evaluated or measured. This occurs especially in children, since they are prone to a high prevalence of routine exposure to disease-causing agent especially in the rural setting. Due to this prevalence, the evaluation and comparison of the immunological profile of urban and rural children becomes necessary.

Our finding on heterophilic agglutinin titres is compatible with that of Agwu *et al*. [7] and Adeniyi-Jones [15] who respectively reported that agglutinin activity against tanned sheep red blood cells was present at high titre in a considerable proportion of school age children. Port-Harcourt city is one of the centres with history of environmental pollution due to consequences of oil exploration (gas flaring, black soot etc.) and other anthropogenic activities. Thus, it is not surprising to have observed the hyper-immune response in the sera of the urban subjects.

CONCLUSION

The findings of this study demonstrate the reaction of urban and rural school age children in Rivers state to heterophile antigens as well as their serum immunoglobulins concentrations. Since environmental toxicants may suppress or stimulate immunologic responses of individuals in high-risk conditions, comparative immunological profile and its relationship with specific environmental toxicants will be a needful tool in regulatory/preventive medicine policy formulations.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

All authors have no actual or potential competing financial interest to declare.

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