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Sex Induced Disparity in Serum Level of Heterophile Antibody in Apparently Healthy School-Age Children in Port Harcourt, Rivers State, Nigeria.

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ABSTRACT

Heterophile antibodies are influenced by both genetic and environmental factors, just like other antibodies. Heterophile antibodies, which are produced by some disorders including infectious mononucleosis (IM), have been found to interfere with the results of hormone assays. With respect to age, sex, species, and social status, their serum titre levels vary. Ninety-six (96) children between the ages of five and twelve who appeared to be in good health were chosen at random. Out of the subjects, sixty (60) were males and thirty-six (36) were women. Blood sample was collected by venipuncture of the antecubital vein of the fore arm of children (5-12 years). By employing the double dilution method, sheep red blood cells (SRBCs) were exposed to human sera in order to cause an antigenic challenge. Heterophile antigen titer value of male and female school age children was compared with one-way analysis of varience (ANOVA) and P value less than 0.05 was considered significant. The results shows that children of between the ages of 5-12yrs possesses heterophile antigens on their red blood cells (RBC) and that this heterophile antigen were observe more on male subjects than female subjects. Hower, the difference between the mean titers of male and female subjects was not statistically significance at P < 0.05. A statistically non-significant difference was observed between heterophile antigen titre of male and female school age children in Port Harcourt metropolis.

INTRODUCTION

Common antigens (or antigens of similar nature) that are possessed by a variety of phylogenetically unrelated species are known as heterophile antigens. Heterophile antigen induced the synthesis of heterophile antibodies. These antibodies can react with certain antigens, which are quite different from, and phylogenetically unrelated to the one which evoked antibody response. Heterophilic antibodies are endogenous, nonspecific antibodies that bind to a variety of different antigens [8].

They can be found in patients with autoimmune diseases, but can also be present in other inflammatory diseases as well as in healthy individuals [1].

Chemically, heterophile antigens are composed of lipoprotein-polysaccharide complexes. There is a possibility of there being identical chemical groupings in the structure of mucopolysaccharides and lipids [4,5,9].

Studies with heterophilic monoclonal antibodies with human tissues shows that humans carry heterophile antigen and are located in different tissues such as the stomach, pancreas, heart, kidney tissue etc. Also, the distribution of binding antigens of each strain of antibodies was different in the population.

This may be the reason why some people are more susceptible to infection with the same pathogenic microorganism [2].

Furthermore, several evidence supports the notion that sex chromosomes and gonadal hormones modulate the number and functions of immune cells. There are well characterized sex differences in the innate and adaptive immune response; there is strong evidence that type I and type II interferon signaling and humoral responses are greater in females than in males across diverse species. Sex differences in both innate and adaptive immunity contribute to the increased prevalence of autoimmunity in females and increase the propensity of females to reject their organs post-transplantation. Sex differences research is uncovering novel therapeutic pathways that could be targeted to improve disease outcomes in all sexes [2]. Thus, the need for this study.

MATERIALS AND METHOD

Research Population and Study Location

Ninety-six (96) apparently healthy children between the ages of 5-12 years were randomly selected. Thirty-six (36/37.5%) of the subjects were female and sixty (60/62.5%) males. The subjects were randomly drawn from government secondary schools, Port Harcourt, Rivers State. The study was carried out within a period of five weeks at the Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Science, University of Port Harcourt.

Ethical Adherence

The University of Port Harcourt Research Ethics Committee granted ethical permission.
The subjects, parents, and guardians were given consent letters in which the goals, procedures, and advantages of the study were spelled out in detail. The consent of parents or guardians was obtained for the collection of blood samples from their children or wards, and their individuals' identities were kept private [6].

Preparation of Sheep's Corpuscle's Preservative (Alsever's Solution)

The salts were measured out using a chemical weighing balance (Mettlo Toledo-PL 2003) and the solution was prepared by mixing 20.5g dextrose, 8g sodium citrate, 0.552g citric acid, and 4.2g sodium chloride in a little amount of distilled water and made up to 1000mL. Then the pH of the solution was adjusted to 6.1. The solution was then divided into aliquots of 25mL, 25mL, and 150 mL and shared into 3 conical flasks respectively. The solutions were autoclaved for 20 min at a pressure of 517mmHg for sterilization and stored at a temperature of 4 ºC.

Collection of Sheep Red Blood Cells

The properly feed sheep was held very firm in a place and furs at the tibial vein region was shaved, ensuring that the tibial veins are visible and the area swabbed with cotton wool soaked in methylated spirit .A 25 G vertinary needle is inserted into the tibial vein and the (25ml) of venous blood from the sheep is drawn into the aliquot bottle containing alservers solution making the solution up to (50ml).The conical flask containing the mixture (alservers solution) was then covered with a cling film and carefully shake and stored in a refrigerator to age (3 days).

Experimental Design

Blood sample was collected by venipuncture of the antecubital vein of the fore arm of children (5-12 years). Antigenic challenge was induced by exposing the human sera to sheep red blood cells (SRBCs) using the double dilution technique.

Assay of Heterophile Antibodies

The anticoagulant known as Alsever's solution was used to preserve SRBCs. To separate the SRBCs from the anticoagulant, this was spun for five minutes at 3000 rpm. To obtain a clean supernatant, SRBCs were spun three times after being cleaned in regular saline. Two drops of regular saline were added to each well in the hemagglutination tray (including the control wells) using a smooth-edged Pasteur pipette. Two drops of the test sample (human serum) were introduced to the ninth well (positive control), and two more drops were added to the

first well and thoroughly mixed. Care was taken in order to reduce bubbles. Two drops of the mixture were transferred from the first well to the second, then from the second well to the third, and so on, up to the eighth well (1/256), in order to make dilutions. Following thorough mixing in the eighth and ninth wells, two drops were pipetted and discarded to provide an equivalent volume of diluted serum. This process was carried out again for every tray that was available. Every well, including the control wells, had two drops of 1% SRBCs added to it, which included doubling dilutions of the test samples. The samples were kept overnight at 4 ºC after being blended to homogeneity by rocking and covered with cling film to avoid evaporation. The titre values for the antigen-antibody reaction were displayed in the agglutination-filled wells. Results were taken by viewing the wells with mat appearance (just like the positive control) as positive (+) while those with button appearance (just like the negative control) as negative. The positive reading with the highest dilution of each row of wells was taken as the titre value for that serum samples [4].

RESULTS

The results shows that children of between the ages of 5-12yrs possesses heterophile antigens on the red blood cells (RBC) and that this heterophile were observe more on male subjects than female subjects. Hower, the difference between the mean titers of male and female subjects was not statistically significance at $P < 0.005$ as shown in Table 1.

Table 1. Heterophile antigen titres and age of male and female apparently healthy school age children

Statistics / Parameters	Sample size (N)	Mean ± SD of titre value	Mean ± SD of subjects age
Female (5-12yrs)	36 (37.5%)	30.83±49.279	7.42±2.383
Male (5-12yrs)	60 (62,5%)	53.20±89.296	8.47±2.288

DISCUSSION

The innate and adaptive immune systems exhibit well-documented sex-based variations; type I and type II interferon signaling as well as humoral responses are clearly higher in females than in males across a wide range of animals. Innate and adaptive immunity differs between the sexes, this is clearly in the likelihood that autoimmunity will develop more often in females than males and transplanted organ rejection is observed more in females. Research on gender differences is identifying new therapeutic targets that may be used to enhance disease outcomes in both sexes [2,7].

There are variations among species in the distribution of heterophilic antigens. Using matching tissue microarrays from other animal species, immunohistochemical staining of fourteen (14) heterophilic antibodies that reacted with antigens in human stomach, pancreas, and kidney tissues showed that these antibodies likewise exhibited distinct response features. These findings revealed that distinct individuals had distinct distributions of heterophilic antigens, which could account for why some people who are infected with the same harmful bacteria become ill while others do not [2,3]. Also, research reports identified different animals, organ systems, ages, environmental dispositions, and sexes to have distinct immune responses. Different species' neuro-immuno-endocrine pathways are influenced by the sex-determining factor of their chromosomes [2,3]. Although the differences between the heterophile antigen titres of male and female school-age children were not statistically significant at $P<0.05$, this could be the cause of the disparities. A larger sample size study will better address this problem.

CONCLUSION

A statistically non-significant difference was observed between heterophile antigen titre of male and female school age children in Port Harcourt metropolis.

Conflict of Interest:

No conflict of interest in this research report.

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