

Levels of serum immunoglobulins in apparently healthy children and adults in Port Harcourt, Nigeria

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Summary: Serum levels of the immunoglobulins: IgG, IgA and IgM were determined by the single radial immunodiffusion technique of Mancini in a total of 122 apparently healthy subjects consisting of 43 children (20 males; 23 females) aged 5-12 years and 79 adults (39 males; 40 females) aged 18-65 years resident in Port Harcourt, southeastern Nigeria. The effects of age and sex on the serum levels of the various immunoglobulin types were also determined. The mean values and ranges for the various immunoglobulin types are presented for both children and adults. Although, female children were found to have significantly lower mean values of IgA compared to male children ($p < 0.05$), there were no significant differences in the mean values of both IgG and IgM between male and female children involved in the present study ($p > 0.05$). However, female adults had significantly higher mean values of IgG and significantly lower mean values of IgA compared to male adults ($p < 0.05$); suggesting a significant gender difference in the value of both IgG and IgA amongst adult subjects. No significant differences were observed in the mean values of any of the various types of immunoglobulin between adults and children. The present study reports mean values of the various types of immunoglobulin in apparently healthy children and adults resident in Port Harcourt, southeastern Nigeria. Significant gender differences were observed for some of the immunoglobulin types only amongst adults. Our results suggest that these gender differences amongst adults were apparently a gradual build-up from childhood. The results also confirm suggestions that levels of some immunoglobulin types seen amongst African adults may have possibly been attained during childhood. Our study could be of value since previous reports in this regard have been relatively scanty especially in this part of Nigeria.

Keywords: Immunoglobulin, Immunity, IgA, IgG, IgM

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Manuscript Accepted: February, 2013

INTRODUCTION

The serum immunoglobulins (Ig) are of potential clinical value in the evaluation of immunoinflammatory diseases and the detection and characterization of immunodeficiency or hypergammaglobulinaemia (Cassidy *et al.* 1974); indeed their uses are extensive, both as diagnostic indicators of disease progression or to monitor therapy, in a variety of hypo- and hypergammaglobulinaemic states (Madison *et al.* 1975).

Several studies have attempted to establish normative values of the various types of serum immunoglobulin for several ambient populations and determine the effects of a number of physiological and other environmental variables on these values. To date, only few studies have established values of human serum immunoglobulin in units compatible with those described by the WHO International Reference Preparations for Human Immunoglobulin (Madison *et al.* 1975); thus confirming suggestions

that, like many haemorrhological parameters, several endogenous and exogenous factors could influence the levels of serum immunoglobulins in human populations (Buckley and Dorsey 1970; Madison *et al.* 1975; Dapper *et al.* 2009). Interplay of several physiologic, genetic and environmental factors is proposed as responsible for the normal adult serum immunoglobulin levels (Cassidy *et al.* 1974). For instance, the effects of age, sex and ethnic origin have been fairly well documented (Butterworth *et al.* 1967; Buckley and Dorsey 1970; Buckley and Dorsey 1971; Cassidy *et al.* 1974; Madison *et al.* 1975; Dapper *et al.* 2009); in addition to the effects of nutrition, infection, geographic location (Madison *et al.* 1975) and altitude (Oyeyinka *et al.* 1982). Normal levels of serum immunoglobulins have long been established for apparently healthy Caucasian children and adults (Stoop *et al.* 1967; Kalff 1970), in normal Indian adults from Kashmiri (Bhat *et al.* 1995) and in a West African (Gambian) community (Rowe *et al.* 1968). Cassidy *et al.* 1974 have shown that in Caucasians the

levels of both IgG and IgA increase with age. Further, slight but significant gender differences in the values of the various immunoglobulin types have been observed: females have higher levels of both IgG and IgM and lower IgA levels compared to males (Cassidy *et al.* 1974). A report by Madison *et al.* 1975, described significant interactions between race, age and sex factors for three immunoglobulin types: IgG, IgA and IgM. However, gender differences apparently depend on the population under investigation as Bhat *et al.* 1995 report no significant differences in the mean serum immunoglobulin levels between sexes, except for the values of IgM which were significantly higher in females compared to males. Several investigators have reported varying levels of serum immunoglobulins amongst different ambient population groups (McFarlane *et al.* 1970; Buckley and Dorsey 1971; Apampa *et al.* 1980; Oyeyinka *et al.* 1982; Oyeyinka *et al.* 1984; Bhat *et al.* 1995). Amongst Africans, various reasons have been adduced for the higher levels of gamma globulins compared to Caucasians; further that West African (Gambian) children attained adult levels of IgG at about the fifth year of life (Rowe *et al.* 1968).

Studies reporting levels of serum immunoglobulins in southeastern Nigerians have been relatively scanty. Most recent reports from Nigeria have focused on immunoglobulin levels in various disease conditions including: diabetes (Akinlade *et al.* 2004), chronic sinusitis (Ogunleye and Arinola 1998), nephrotic syndrome (Oviasu *et al.* 1998), schistosomiasis (Arinola and Salimonu 1999), various types of cancer (Olubuyide *et al.* 1993; Opkala and Salimonu 1994) and in pre-eclampsia (Okerengwo *et al.* 1990) but have emanated mainly from southwestern Nigeria. To our best knowledge, no previous reports have documented serum immunoglobulin values from Port Harcourt, an important center for the petro-chemical industry in the Niger Delta region of Nigeria. Such a report has become necessary following the rapid urbanization of Port Harcourt, the capital of Rivers State due to recent influx of multinational concerns involved with petroleum exploitation and exploration (Dapper *et al.* 2008). The present study attempts to establish normative values for the various serum immunoglobulin types for apparently healthy children and adults in Port Harcourt, Nigeria and further, determine the possible effects of various physiological factors on the levels of the various types of immunoglobulins amongst our population. This is part of a series of ongoing studies from our center aimed at establishing reference ranges and normative values for various haemorheological and immunological parameters in our environment (Dapper *et al.* 2008; Dapper *et al.* 2009).

MATERIALS AND METHODS

Subjects: A total of 122 subjects were randomly recruited into the study. This consisted of 43 children (20 males and 23 females) aged between 5 and 12 years and 79 adults (39 males and 40 females) aged

between 18 and 65 years. All subjects were resident in Port Harcourt, Nigeria. Subjects were from various socio-economic classes and ethnic groups in southeastern Nigeria. The children were drawn mainly from primary schools in Port Harcourt. No subject had antecedent history of haematologic, endocrine or metabolic disease or was on any medication likely to influence any of the immunological parameters under investigation. Informed consent was sought and obtained from each adult subject and from the parent or guardian of each child before recruitment into the study.

Blood collection: 3-5 ml of venous blood was carefully collected from each subject using sterile disposable syringes from a peripheral vein commonly an ante cubital vein. The procedure was explained to each subject to enhance cooperation and carefully done to avoid stasis. Each child was well reassured all through the procedure. The blood was immediately transferred to sample bottles containing EDTA and carefully mixed. All blood samples were collected between 9am and 12noon each day. The collected blood was centrifuged to obtain the serum which was then stored at -20°C until ready for immunoglobulin assay. Assay was done within 2 weeks of blood collection.

Methods:

Quantitative determination of serum immunoglobulin values of subjects

This was done using the single radial immunodiffusion method of Mancini *et al.* 1965 using locally prepared plates. This method is based on the formation of a ring of precipitate between an antibody in an agar gel and an added antigen. Briefly, after the preparation of the phosphate buffer solution (PBS 7.2) commercially obtained agar was next dissolved using a Bunsen burner. 1.5ml of human anti-sera (i.e. IgA, IgG and IgM) was mixed with the 5.0ml of the phosphate buffer solution and both were mixed with 7ml of the dissolved agar in test tubes. The mixture was next poured unto glass slides on a leveler and allowed to congeal after which they were stored in a moist chamber. The agar plate was placed on a template and a suction pump used to bore holes (wells) on the agar. Serum from each subject was next added to the wells for each of the immunoglobulins. After this procedure the plates were stored in a moist box at -4°C for a period of standard hours: 4hours for IgG and 18hours for both IgA and IgM. The diameter of the rings that occur after their respective time frames for each well were measured with a scientific rule and recorded. A graph of the standard concentrations on the ordinate was plotted against their standard diameter on the abscissa axis using a semi-log paper. The concentrations in the

test solutions were obtained by reading the concentration of standard indicated by the ring diameter of the test solution. The standards were read and calculated and used as a basis for the diameters measured from the wells containing the serum samples of each subject.

Statistical Analysis

The results obtained are presented in Tables 1, 2 and 3. Statistical differences between sexes and between adults and children were determined using the students’ t-test: a value of $p < 0.05$ was considered statistically significant.

RESULTS

Table 1 shows the values of the various immunoglobulin types studied for children. The mean IgG, IgA and IgM values for male children were found to be 877.15 ± 97.80 mg/dl; 525.99 ± 97.26 mg/dl and 18.66 ± 2.10 mg/dl respectively; the corresponding values for female children: 971.36 ± 103.06 mg/dl; 257.84 ± 43.09 mg/dl and 22.91 ± 2.75 mg/dl for IgG, IgA and IgM respectively. Significant gender differences were observed only in the values of IgA amongst children: male children were found to have significantly higher values of IgA compared to female children ($p < 0.05$). There were no significant differences in the mean values of the other immunoglobulin types amongst children although female children were found to have higher values of IgG. Differences in IgM between female and male children were marginal. All the same, values of the various immunoglobulin types for children were pooled together and are as presented in Table 1 for the Total children population.

Table 2 shows the values of the various immunoglobulin types studied for the adult population. The mean IgG and IgA values for adult males were found to be 799.89 ± 29.27 mg/dl and 596.69 ± 80.31 mg/dl respectively. Adult male IgG values were found to be significantly lower, while adult male IgA values were found to be significantly higher than the corresponding values for adult females which were 971.36 ± 42.80 mg/dl and 316.70 ± 37.54 mg/dl for IgG and IgA respectively ($p < 0.05$). Although, no statistically significant gender differences were observed in the mean values of IgM between adult males and adult females which were 21.94 ± 2.31 mg/dl for males and 18.29 ± 1.41 mg/dl for females respectively ($p > 0.05$); adult male values were found to be marginally higher.

Table 3 shows the values of the various immunoglobulin types for all subjects involved in the present study. The mean IgG, IgA and IgM values for all adult subjects were found to be 886.70 ± 27.64 mg/dl; 451.24 ± 45.84 mg/dl and

20.09 ± 1.35 mg/dl respectively. The corresponding mean IgG, IgA and IgM values for all children were found to be 920.97 ± 77.62 mg/dl; 407.51 ± 59.15 mg/dl and 20.64 ± 1.71 mg/dl respectively. No significant differences were observed in the mean values of all types of the various immunoglobulins studied between adults and children ($p > 0.05$); although the mean values for IgG were higher in children and the mean values for IgA higher in adults. Differences in the mean values of IgM between adults and children were marginal. On account of the non-significant differences in the mean values of the various immunoglobulins, the results obtained were therefore pooled to obtain values for the Total population including children and adults and are as presented in Table 3.

Table 1. Values of the various immunoglobulin types for apparently healthy children in Port Harcourt, Nigeria

| Type | Total children (n=43) | Male children (n=23) | Female children (n=20) |
|-------------|--------------------------------|--------------------------------|---------------------------------|
| IgG (mg/dl) | 920.97±77.62 (467.0-2708.6) | 877.15±97.80 (467.0-2708.6) | 971.36±103.06 (467.0-2708.6) |
| IgA (mg/dl) | 407.51±59.15 (11-1410.0) | 525.97±97.26 (47.0-1410) | 257.84±43.09* (10.9-526.40) |
| IgM (mg/dl) | 20.64±1.71 (8.28-48.3) | 18.66±2.10 (8.28-40.20) | 22.91±2.75 (8.28-48.30) |

All values=Mean ± SEM; ranges in parenthesis * $P < 0.05$, male Vs Female

Table 2. Values of the various immunoglobulin types for apparently healthy adults in Port Harcourt, Nigeria

| Type | Adult males (n=39) | Adult females (n=40) |
|-------------|----------------------------------|-----------------------------------|
| IgG (mg/dl) | 799.89±29.27 (467.00-1307.60) | 971.36±42.80* (653.80-1868.00) |
| IgA (mg/dl) | 596.69±80.31 (5.30-1410.00) | 316.70±37.54* (5.30-1410.00) |
| IgM (mg/dl) | 21.94±2.31 (8.28-69.00) | 18.29±1.41 (8.28-40.02) |

All values=Mean ± SEM; ranges in parenthesis

Table 3. Values of the various immunoglobulin types for all subjects in Port Harcourt, Nigeria

| Type | Total population (n=122) | Total adults (n=79) | Total children (n=43) |
|-------------|--------------------------------|-------------------------------|--------------------------------|
| IgG (mg/dl) | 898.75±32.53 (467.0-2708.6) | 886.7±27.64 (467-1868.0) | 920.97±77.62 (467.0-2708.6) |
| IgA (mg/dl) | 434.03±36.21 (513.0-1410.0) | 451.24±45.84 (5.30-1410.0) | 407.51±59.15 (11-1410.0) |
| IgM (mg/dl) | 20.28±1.06 (8.28-69.0) | 20.09±1.35 (8.28-69.00) | 20.64±1.71 (8.28-48.3) |

All values=Mean ± SEM; ranges in parenthesis

DISCUSSION

The present study reports for the first time, normative values of some immunoglobulin types: IgG, IgA and IgM amongst apparently healthy children and adult residents of Port Harcourt, southeastern Nigeria; previous studies in this regard from Nigeria have emanated from the southwestern and northern parts of

the country and were published about three decades ago (Apampa *et al.* 1980; Oyeyinka *et al.* 1982; Oyeyinka *et al.* 1984). For our adult population, the values of the various immunoglobulin types obtained in the present study are at variance with values previously obtained for adults of both Caucasian (Madison *et al.* 1975; Stoop *et al.* 1967) and Negro (Madison *et al.* 1975) origin: being generally lower for IgM, higher for IgA, but are of the same range for IgG. The present study also reports values of the various serum immunoglobulins generally higher than values reported previously in both Jamaicans and Nigerians (McFarlane *et al.* 1970). Reasons for this pattern of differences are not immediately clear but perhaps may be related to a number of the factors described above (Butterworth *et al.* 1967; Stoop *et al.* 1967; Buckley and Dorsey 1970).

The present study reports significantly higher levels of IgG in adult females compared to adult males. This is consistent with the report of Cassidy *et al.* 1974 but not with that of Bhat *et al.* 1995, the later describe non-significant gender differences in some serum immunoglobulin levels. The present study also reports significantly lower IgA values in female adults compared to male adults consistent with the report of Cassidy *et al.* 1974 in Caucasians. Further, the non-significant gender differences in the values of IgM found in the present study is consistent with most previous reports (Stoop *et al.* 1967; Weeke 1968; Buckley and Dorsey 1970; Kalff 1970; Buckley and Dorsey 1971; Cassidy *et al.* 1974).

Although, non-significant differences were observed in the mean values of both IgG and IgM amongst children involved in the present study; females were found to have significantly lower IgA levels, non-significant but consistently higher IgG levels and marginally higher IgM levels compared to males. Our result suggests that amongst our childhood population, this pattern of differences apparently persists unto adulthood where initial childhood non-significant differences in IgG values indeed later become significant. Amongst Caucasian children, gender differences have previously been reported by Stoop *et al.* 1969; with females having significantly higher IgM and IgG levels compared to males. The non-statistically significant gender differences in the mean values of both IgG and IgM seen in the present study is consistent with the report of Rowe *et al.* 1968 who also describe non-significantly higher mean values of IgM in female as compared to male children. Marginal differences in IgA levels amongst male and female children seen in the present study is consistent with the report of Stoop *et al.* 1969.

From the results of our study many factors apparently do contribute to the adult levels of the various immunoglobulins. Our study confirms earlier

reports that sex and age may affect levels of the various immunoglobulins. The adult mean values of IgG and IgA seen the present study compares with reported values by Bhat *et al.* 1995 in an adult population from Kashmiri, India, however, values for IgG and IgA obtained from the present study are apparently higher than reported values by Oyeyinka *et al.* 1984 amongst Nigerians in Ibadan, in southwest Nigeria and in Jos and Zaria, both in northern Nigeria. However, values of IgM seen in the present study appear lower than reported values by Oyeyinka *et al.* 1984. Precise reasons for these differences are not immediately clear; ethnic, geographic location and perhaps the cumulative effects of petroleum exploration and exploitation in the Port Harcourt environment could be contributory factors; as well as the three decade time interval between these earlier reports and the present.

In conclusion, the present study reports for the first time, serum levels of immunoglobulin G, A and M in apparently healthy children and adults resident in Port Harcourt, southeastern Nigeria. Gender differences were found in the values of some immunoglobulin types with adult females having significantly higher mean values of IgG and lower mean values of IgA compared to male adults ($p < 0.05$); Similar gender differences were observed amongst children only for values of IgA. The results also confirm suggestions that levels of some immunoglobulin types seen amongst African adults may have possibly been attained during childhood (McFarlane *et al.* 1970). Our findings confirm previous suggestions that levels of serum immunoglobulin in humans are age and sex dependent and may vary with ethnic background and geographical location even within a particular country. Our study could be of value since previous reports in this regard especially from this part of Nigeria have been relatively scanty.

Acknowledgement

We would like to thank Professor OG Arinola of the University of Ibadan, Nigeria for his technical assistance during the course of this study.

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