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Complement and immunoglobulin levels in Ilorin Nigeria, and environ

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Summary

The levels of complement components C3 and C4 were determined in EDTA (ethylene-diamine-tetra-acetic acid) plasma of 106 apparently healthy people aged 11-50 years, and in 23 paired maternal and cord plasma in Ilorin. The immunoglobulins G, A and M concentrations of these paired samples were also assayed. Furthermore, serum IgG, IgA and IgM levels were quantitated in 501 apparently healthy residents of Ilorin (1-84 years old, n=313) and its suburban community, Shao (11-80 years old, n=188).

Generally, there were no significant sex-related differences in C3 and C4 values, but these values increased steadily with age. C3 and C4 levels in cord plasma were high, representing 73% and 88% of adult levels respectively. Maternal levels of these complement components were higher ($P < 0.02$) than values obtained in non-pregnant females of comparable age. Immunoglobulin G values obtained for maternal and cord samples compare well with figures reported previously from other centres in Nigeria. Only 1 of 23 IgA and 2 of 23 IgM samples showed detectable values of these immunoglobulins in cord blood.

The mean concentrations of immunoglobulins obtained were similar for IgG and lower for IgA and IgM in Ilorin, but higher for IgG and lower for IgA and IgM in Shao, when compared with data reported from Enugu and Zaria — both in Nigeria. Shao showed significantly higher mean level of IgG but similar levels of IgA and IgM to values obtained in Ilorin. Generally, IgG and IgA concentrations rose

gradually up to age group 31-40 and 41-50 years respectively; and then declined. There was no consistent rise in IgM levels in the age groups studied. Except for IgA and IgM values in Shao people age 21-30 years and over 50 years respectively, no statistically significant sex differences could be established for IgG, IgA and IgM ($P > 0.05$ in all other cases) in this study. However, the levels of these three immunoglobulins were in general higher in females than in males.

Résumé

Les niveaux de composants complémentaires C3 et C4 ont été déterminés par le plasma EDTA (l'acide éthylène-diamine-tétra-acétique) de 106 personnes de bonne santé ayant l'âge de 11 à 50 ans, et de 23 plasma maternel et plasma de cordon accouplés à Ilorin. Les immunoglobulines G, A et des concentrations M de ces échantillons couplés ont été essayés. En plus, les niveaux de serum IgB IgA et IgM ont été déterminés pour 501 habitants apparemment de bonne santé, à Ilorin âgés de 1-84 ans totalisant 313 et sa banlieue, Shao de 11-80 ans totalisant 188.

En général, il n'y avait pas de différence significative relative au sexe des valeurs C3 et C4, mais ces valeurs accroissent régulièrement avec l'âge. Les niveaux C3 et C4 de plasma de cordon étaient élevés représentant 73% et 88% de niveaux adulte. Les niveaux maternels de ces composants complémentaires étaient plus élevés ($p < 0.02$) que des valeurs obtenues chez les femmes non-enceintes de l'âge comparable. Les valeurs de l'immunoglobuline

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G obtenues pour des échantillons de plasma maternel et de plasma de cordon comparant favorablement aux chiffres au Nigéria. Sauf I des 23 échantillons IgA et 2 de 23 IgM ont des valeurs visibles des immunoglobulines du sang de cordon.

La moyenne des concentrations d'immunoglobulines obtenue ici est pareille à IgG et peu élevée pour IgA et IgM à Ilorin, mais plus élevée pour IgG et moins pour IGA et IgM à Shao en comparaison aux données rapportées d'Enugu et de Zaria au Nigéria. Shao a démontré un niveau de moyenne élevée, et significative de IgG mais des niveaux semblables de IgA et IgM en comparaison aux valeurs obtenues à Ilorin.

En général les concentrations IgG et IgA ont accru progressivement jusqu'aux groupes d'âge de 31-40 et de 41-50 respectivement; et puis ont baissé. Il n'y avait pas de hausse régulière aux niveaux IgM dans tous les groupes d'âge étudiés. Sauf pour les valeurs IgA et IgM de personnes à Shao âgées de 21 à 30 ans et plus de 50 ans, on ne pourrait pas établir statistiquement des différences des sexes significatives pour IgG et IgA et IgM ($p > 0.05$ dans tous les autres cas) dans cette étude. Cependant les niveaux de ces trois immunoglobulines étaient en général plus élevés dans la femelle que dans le mâle.

Introduction

The study of immunoglobulin levels in healthy Nigerians has received a lot of attention over the years. Adult values have been reported by many workers[1-4]; infants and neonates have been well investigated[5-7], and foeto-maternal concentrations have also been recorded[8]. In contrast to the situation with the immunoglobulins, information on the levels of complement proteins in healthy Nigerians is scanty. Abdurrahman *et al.*[6] reported relative C3 levels only in the first year of life in healthy Nigerian children. Other reports have monitored changes in the relative concentrations of complement components in various diseases[9-14]. In all cases, samples representing the normal or control groups were few, of one sex or limited to narrow age groups. The present study reports the quantitative concentrations of C3 and C4 in apparently healthy Nigerians of both sexes and of wider age range (newborn and 11-50 years). Although IgG, IgM and IgE levels of a few control individuals from Apado, about 45 KM from Ilorin, had been investigated[15], this study also documents the first comprehensive report of serum

immunoglobulin levels in healthy people in Ilorin and environ.

Subjects and methods

A total of 547 blood samples from people resident in Ilorin and Shao were studied. Ilorin is the capital city of Kwara State, Nigeria and Shao lies about 10KM north of it. 106 of the samples (39 males, 67 females; aged 11-50 years) came from students and teachers of Ilorin Teachers College (ITC) Ilorin. These were collected in disodium-EDTA anticoagulant (20 mM final concentration) and plasma was obtained as reported previously[16]. 23 paired maternal and cord plasma were obtained by similar treatment immediately after child birth at the labour ward of the University of Ilorin Teaching Hospital, Ilorin. Immediately after delivery of the baby, the cord was clamped twice and divided. Prior to delivery of the placenta cord blood sample was obtained by releasing the cord clamp, avoiding contamination with maternal blood. The mothers were aged 17-40 years (27 ± 5 years) and the babies had a mean gestational age of 36 ± 2 weeks (range 32-38 weeks). The other samples were sera obtained from University of Ilorin staff and their families who supplied 207 of these samples (108 males, 99 females; aged 1-84 years) while 188 samples (103 males, 85 females; aged 11-80 years) came from residents of Shao. They are predominantly farmers. The plasma and sera obtained were stored at -20°C until assayed.

Plasma C3 and C4, and immunoglobulins G, A and M levels were measured by single radial immunodiffusion technique[17] using commercial monospecific antisera to human C3c, C4, IgG, IgA and IgM respectively (Serotec, Oxford OX5 1JE, England). The levels were measured against a commercial serum standard (Kontroll- Plasma-human-lot No. 04 7009; Behringwerke AG, Marburg, W. Germany), and are expressed in g/l. The levels of C3 and C4 were also measured against a pooled EDTA plasma standard obtained from 10 adults of age 21-30 years and expressed as percentage. Statistical analysis was by student's t-test.

Results

All the samples for C3 and C4 assay were run in a single batch and intra-batch coefficient of variation (c.v.) was 13% for C3 and 6% for C4. The inter-batch c.v. of the assay methods were 3% for IgG, 13% for IgA and 6% for IgM.

Tables 1 and 2 show plasma C3 and C4 levels respectively in people aged 11-50 years in Ilorin. Significant difference between males and females existed only in the 11-20 years age group for C3 and only in the 21-30 years age group for C4. Generally, there were a gradual increase in C3 and C4 concentrations with rising age. Mean C3 and C4 concentrations in males expressed as percentage of the pooled local plasma standard were $137 \pm 42\%$ and $123 \pm 30\%$ respectively for the 21-30 years age group.

Table 1: Plasma C3 levels (mean \pm s.d. and range in g/l) in Ilorin

| Age (years) | Males + Females | Males | Females |
|-------------|--|--|--|
| 11 - 20 | 0.45 \pm 0.15 (0.11 - 0.74) n=40 | 0.39 \pm 0.10 (0.24 - 0.59) n=15 | 0.48 \pm 0.17 (0.11 - 0.74) n=25 |
| 21 - 30 | 0.48 \pm 0.14 (0.25 - 0.74) n=38 | 0.43 \pm 0.13 (0.25 - 0.68) n=13 | 0.51 \pm 0.14 (0.28 - 0.74) n=25 |
| 31 - 40 | 0.54 \pm 0.18 (0.27 - 0.94) n=18 | 0.56 \pm 0.17 (0.41 - 0.85) n=7 | 0.53 \pm 0.20 (0.27 - 0.94) n=11 |
| 41 - 50 | 0.52 \pm 0.18 (0.34 - 0.94) n=10 | 0.54 \pm 0.09 (0.41 - 0.59) n=4 | 0.51 \pm 0.22 (0.34 - 0.94) n=6 |

n is sample number. *Significant difference between males and females ($P < 0.05$). The mean values were not significantly different among the various age groups ($P > 0.05$) except for 11-20/31-40 and 11-20/41-50 comparisons in the male ($P < 0.05$).

Table 2: Plasma C4 levels (mean \pm s.d. and range in g/l) in Ilorin

| Age (years) | Males and females | Males | Females |
|-------------|--|--|--|
| 11 - 20 | 0.19 \pm 0.05 (0.12 - 0.30) n=40 | 0.20 \pm 0.06 (0.12 - 0.30) n=15 | 0.19 \pm 0.04 (0.13 - 0.27) n=25 |
| 21 - 30* | 0.25 \pm 0.14 (0.13 - 0.85) n=38 | 0.20 \pm 0.05 (0.13 - 0.28) n=13 | 0.28 \pm 0.16 (0.13 - 0.85) n=25 |
| 31 - 40 | 0.26 \pm 0.13 (0.16 - 0.76) n=18 | 0.28 \pm 0.21 (0.17 - 0.76) n=7 | 0.24 \pm 0.05 (0.16 - 0.32) n=11 |
| 41 - 50 | 0.28 \pm 0.09 (0.16 - 0.41) n=10 | 0.34 \pm 0.06 (0.27 - 0.41) n=4 | 0.24 \pm 0.09 (0.16 - 0.41) n=6 |

n is sample number. *Significant difference between males and females ($P < 0.05$). Significant difference ($P < 0.05$) in mean values among the various age groups were found only in the following comparisons: 11-20/21-30, 11-20/31-40, 11-20/41-50 in the combined samples; 11-20/41-50, 21-30/41-50 in the male samples; and 11-20/21-30, 11-20/31-40, 11-20/41-50 in the female samples.

Complement and immunoglobulin levels for maternal and cord plasma are shown in Table 3. Cord plasma C3 and C4 values were high (0.35 ± 0.09 /l and 0.22 ± 0.05 /l respectively) and represented $63 \pm 29\%$ for C3 and $42 \pm 14\%$ for C4, of maternal concentrations (0.62 ± 0.17 /l and 0.58 ± 0.24 /l respectively) of these proteins. The cord plasma

values measured 73% and 88% of adult levels (21-30 years age group, Tables 1 and 2) of C3 and C4 respectively. The differences in the levels of C3 and C4 in maternal plasma (which was higher) compared to non-pregnant adult females (Table 4) are significant ($P < 0.02$ in both cases).

Table 3: Complement and immunoglobulin levels (mean \pm 1s.d. and range in g/l) in maternal and cord plasma in Ilorin

| Component | Maternal | Cord | Cord + |
|-----------|--|--|---|
| C3 | 0.62 ± 0.17 (0.37 - 0.94) n=23 | 0.35 ± 0.09 (0.21 - 0.59) n=23 | 63 ± 29 (23 - 160) n=23 |
| C4 | 0.58 ± 0.24 (0.21 - 1.26) n=23 | 0.22 ± 0.05 (0.13 - 0.33) n=23 | 42 ± 14 (18 - 67) n=23 |
| IgG | 21.29 ± 6.95 (9.39 - 36.93) n=23 | 16.86 ± 4.80 (6.17 - 25.81) n=23 | 85.60 ± 33.31 (35.86 - 183.33) n=23 |
| IgA | 2.45 ± 1.84 (1.02 - 10.26) n=23 | 0.25^* — n=1 | 17.44^* — n=1 |
| IgM | 1.48 ± 1.09 (0.51 - 4.98) n=23 | 0.33^* (0.27 - 0.39) n=2 | 31.25^* (10.16 - 52.33) n=2 |

n is sample number. *Cord blood values expressed as percentage of corresponding maternal concentration. *Only 1 sample for IgA (4.2%) and 2 samples for IgM (8.3%) showed detectable levels of these immunoglobulins in cord plasma. The mothers were aged 27 ± 5 years (range: 17-40 years). Mean gestational age of the babies was 36 ± 2 weeks (range: 32-38 weeks).

Table 4: C3 and C4 levels in maternal plasma and in non-pregnant adult female controls

| | C3 $\bar{X} \pm 1S.D.$ | C4 $\bar{X} \pm 1S.D.$ |
|----------|---------------------------|---------------------------|
| Maternal | 0.62 ± 0.17 n=23 | 0.58 ± 0.24 n=23 |
| Controls | 0.51 ± 0.14 n=25 | 0.28 ± 0.16 n=25 |
| P | < 0.02 | < 0.01 |

Mean values are in g/l. The mothers were 27 ± 5 years old and the non-pregnant adult females 26 ± 3 years old. P values were derived from student's t-test. n is sample number.

Table 5: Mean immunoglobulin levels (\pm 1 s.d. and range in g/l) in Ilorin and Shao

| Component | Ilorin | Shao | Students t-test |
|-----------|---|--|-------------------------|
| IgG | 21.75 \pm 9.15 (2.69 - 67.09) n=311 | 25.78 \pm 8.51 (10.51 - 60.31) n=185 | t = 4.958 (P < 0.01) |
| IgA | 1.60 \pm 0.83 (0.27 - 5.85) n=305 | 1.55 \pm 0.84 (0.40 - 5.85) n=183 | t = 0.639 P > 0.20 |
| IgM | 1.08 \pm 0.69 (0.27 - 5.82) n=292 | 1.19 \pm 0.79 (0.39 - 6.75) n=177 | t = 1.532 P > 0.10 |

Mean values expressed here cover people (males and females) aged 1-84 (26.86 \pm 14.25, n=313) years in Ilorin and 11-80 (39.41 \pm 17.12, n=188) years in Shao. n is sample number.

IgG concentrations obtained for maternal and cord blood (Table 3) revealed that the cord samples contained about 85.6% of maternal values. IgA was detectable in only 1 of 23, and IgM in only 2 of 23, samples. Mean immunoglobulin levels obtained in Ilorin and Shao are shown in Table 5. IgG levels were significantly higher in samples from Shao while IgA and IgM concentrations were the same for the two locations. Figures 1 and 2 show age and sex-related variations in immunoglobulin levels in Ilorin and Shao respectively. Generally, there was a rise in IgG and IgA values with increasing age up to 31-40 years age group for IgG and 41-50 years age group for IgA. Both then declined. No consistent rise in IgM levels could be observed in the age groups studied. Also, no significant sex differences could be established for IgG, IgA or IgM in this study (P > 0.05 in most cases). However, IgA values in the age group 21-30 years and IgM values in the age group > 50 years in Shao showed significant difference with higher levels in females than males. Female values were generally slightly higher than male figures for the three immunoglobulins.

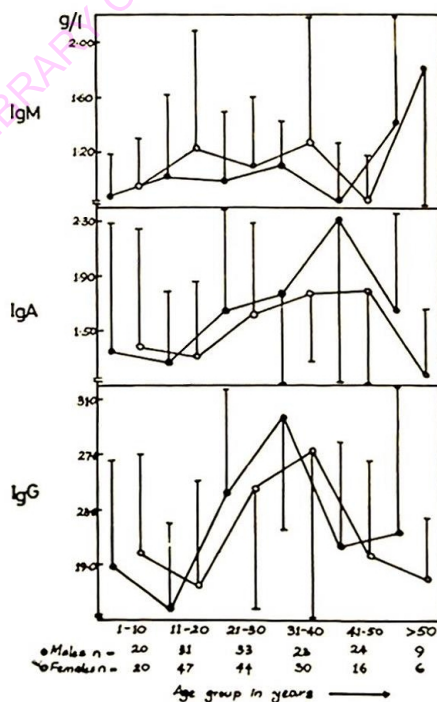


Fig. 1: Mean immunoglobulin levels, in males and females by age, for Ilorin. Each point represents mean \pm 1 s.d.

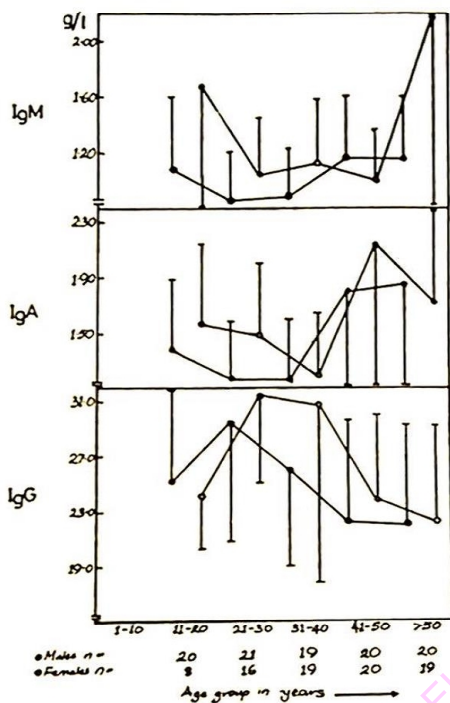


Fig. 2: Mean immunoglobulin levels, in males and females by age, for Shao. Each point represents mean \pm 1 s.d.

Discussion

The main factors contributing to variations in the levels and pattern of serum proteins include parasitic infestation, socio-economic and cultural factors[4]. Edozien[18,19], working at Ibadan, attributed the high gamma-globulin levels in Nigerians to the prevalence of malaria and other microbial infections. Infection and malnutrition are also known to affect the level of some complement components[12]. Our relative figures, obtained when values were derived in comparison with a pooled local plasma standard, showed agreement with previous reports. For instance, the mean C3 and C4 values of $137 \pm 42\%$ and $123 \pm 30\%$ respectively which we obtained for men aged 25 \pm 3 years in the present report are not significantly different from $143.8 \pm 32.5\%$ for C3 and $119 \pm 50\%$ for C4 reported before in normospermic Nigerian men[14]. Our quantitative figures for C3 are lower than values found in normal

healthy subjects in India[20]. The discrepancy may be due to the fact that anti-C3 was used in radial immuno-diffusion technique to quantify the Indian samples. It has been recommended that only anti-C3c should be used in the immunochemical estimation of C3 levels[21]. Environmental factors may also be responsible for the difference. However Salimonu[22] has reported a higher mean C3 level, which is lower than that found in India[20], in Nigeria children aged 1-4 years. The younger age of the children may be responsible for the higher levels compared to this report as C3 levels are high in cord plasma of Nigerian neonates[6]. Differences due to sex were not demonstrable in C3 and C4 levels in this study, but there was a steady rise in values with increasing age.

High levels of cord plasma C3 observed here compared well with the findings of Abdurrahman *et al* [6] in Zaria, Nigeria. A relative mean concentration of $112 \pm 29\%$ of a pooled local plasma standard was obtained here while we reported a mean C3 level of 99% of a pooled local plasma standard at birth in Zaria. C3 and C4 have been detected in the foetus as early as 15 weeks gestation[23], and complement activity within the range found in normal adult sera has been reported in premature neonates[6]. The reason for higher levels of C3 and C4 in maternal samples compared to age-matched non-pregnant females may be due to increased synthesis of these proteins by nursing mothers.

Our mean IgG of $16.86 \pm 4.8\text{g/l}$ (Table 3) in cord plasma is not significantly different from 1.65g/l reported by Adeniyi and Ayeni[5] for Ibadan, Nigeria, but lower than the figure ($260 \text{ i.u./ml} = 22.58\text{g/l}$) for Zaria[6]. Our cord plasma IgG concentration was 85.6% of maternal level while the report of Salimonu and coworkers[8] indicated that their cord sera (from 32-36 weeks gestational age group) measured 79% of maternal concentrations. Detectable levels of IgA and IgM in cord plasma were infrequent in this study. Adeniyi and Ayeni[5] could also not detect IgA at birth in any of the children they studied but low levels of IgM were obtained by them and Salimonu *et al* [8]. Some of the infants studied in Zaria[6] also lacked detectable levels of IgM. The maternal concentrations of IgG, IgA and IgM reported here are similar to those reported previously[8] from Ibadan, Nigeria, in mothers of newborns (32-36 weeks gestational age).

Immunoglobulin concentrations presented in this report for healthy people in Ilorin and Shao are lower

for IgA and IgM in comparison with documented figures for Enugu[4] and Zaria[2]. The mean IgG level for Ilorin is comparable, and the one for Shao is higher than those reported from Enugu and Zaria. The higher IgG levels may be due partly to the fact that Shao is mesoendemic for river blindness[24]. Indeed, Shao showed significantly higher mean concentration of IgG than Ilorin. The decline in IgG levels after 31-40 years age group is consistent with the findings of Buckley and Dorsey[25,26] who described a fall in mean concentrations of IgG after the age of 35 years in North Carolina. Also consistent with their reports and that of Oyeyinka *et al* [3] is the observation that IgA concentrations increased with advancing age up to age group 41-50 years. No consistent elevation in IgM values with advancing age was noted in Ibadan[3] or in this report. The lack of sex differences in immunoglobulin levels observed in this report is similar to the findings of previous workers[3,27,28] for IgA. Females have been reported to have higher IgG[28,29] and IgM[29] levels than males. The higher IgM and IgG levels obtained in females in this report were not significant. This may be due to the higher prevalence and intensity of onchocerciasis for males than for females in Shao[24]. However, this is not likely to be all the explanation as onchocercal infections are accompanied by greater elevations of IgE than of any other immunoglobulin class.

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