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Serum immunoglobulin levels in the course of normal gestation in Nigerian women

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Summary

The three major immunoglobulin (IgG, IgA and IgM) concentrations were measured in 118 Nigerian women during normal pregnancy. IgG was found to decline progressively throughout gestation with a very highly significant decrease between the first and second trimester ($0.01 > P > 0.005$) and between the second and third trimesters ($P = 0.01$). The mean concentrations of IgM were significantly lower in sera from first-trimester pregnant women than those of age-matched non-pregnant control subjects ($P < 0.001$), and there was a further significant decrease between second and third trimester groups ($0.005 > P > 0.001$). No statistical difference was observed for IgA levels throughout normal pregnancy. The significance of these results is discussed in relation to other observations elsewhere.

Résumé

Ses concentrations des trois principales immunoglobulines (IgG, IgA et IgM) eut été mesurées chez 118 Nigérianes pendant la période normale de grossesse. Nous avons constaté que l'IgG avait tendance à baisser progressivement pendant la grossesse, surtout pendant et le deuxième trimestre de la grossesse ($0.01 > P > 0.005$) et pendant le second et le troisième trimestres ($P = 0.01$). La moyenne de la concentration d'IgM était nettement plus basses dans les sérums des femmes ayant une grossesse de trois mois que dans ceux des femmes normales de même âge qui n'attendaient pas de bébé ($P < 0.001$); cette moyenne baisse nettement aussi entre le 6ème et le 9ème mois chez les femmes enceintes ($0.005 > P > 0.001$). Nous n'avons remarqué aucune différence statistique dans le niveau d'IgA pendant toute la durée des grossesses normales. La signification de tous ces constats a été analysée par rapport à d'autres observations faites ailleurs.

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Introduction

The placenta mediates foetal acquisition of passive immunity by allowing the transport of maternal immunoglobulin G (IgG). Studies have shown that the transfer of maternal IgG (but not other immunoglobulin classes) to the foetal circulation is selective [1–4], resulting in lower levels of IgG in pregnant women [5]. There continues to be conflicting reports on changes in serum levels of IgA and IgM, in the course of normal human gestation. A gradual increase in IgM throughout gestation has been reported by McFarlane *et al.* [6] while no significant change was observed by others [7, 8]. The concentration of IgA has also been shown to remain fairly constant throughout gestation [7].

Nigerians, in common with indigenous populations of other tropical countries generally have higher levels of immunoglobulins than Caucasians [9–11]. The pattern of immunoglobulins in pregnant Nigerian women could also differ from those of Caucasians, however, very little information exists in this direction. Earlier work by McFarlane *et al.* [6] was carried out over a decade ago, and since immunoglobulin levels are influenced by the environment, changes in immunoglobulin levels of pregnant Nigerian women would change with improved standard of living. The only recent information

was provided in Ibadan over 300 km from Benin City, and was restricted mainly to immunoglobulin levels at delivery [12,13].

The present study concerns the three major immunoglobulin (IgG, IgA and IgM) concentrations in the sera of normal pregnant women in Benin City, Nigeria.

Subjects and methods

Population studied

Venous blood samples were collected from 118 healthy pregnant Nigerian women, aged 15–35 years, attending the antenatal clinic of the University of Benin Teaching Hospital (UBTH). They consisted of first trimester pregnancies (up to 13 weeks gestation, $n = 19$); second trimester pregnancies (from 14–26 weeks gestation, $n = 48$); third trimester pregnancies (from 27–40 weeks gestation, $n = 33$); and post-partum subjects (within 10 days of delivery, $n = 18$). All women were healthy and had no history of malaria or other infections, and they were all protected throughout gestation with anti-malarial drugs. Controls consisted of 37 age-matched normal non-pregnant female subjects. None of the subjects in the control group used oral contraceptives and their last pregnancy, if any, occurred at least 1 year before the commencement of the study. Thus, a total of 155 serum samples were separated at room temperature after the clot had retracted, and were stored at −20°C until required for analysis.

Immunoglobulin measurement

Concentrations of immunoglobulins were determined by the single radial immunodiffusion method of Mancini as modified by Fahey and MacKelvey [14] using rabbit antisera specific for each of IgG, IgA and IgM (DAKO A/S, Denmark). Equal volumes of optimally diluted antiserum and 2% noble agar (Difco Inc., Detroit, U.S.A.) at 56°C were mixed thoroughly and poured onto agar-coated 10 × 10 cm glass plates (Bio-Rad, Watford, U.K.). Wells of 2 mm diameter 1 cm from each other were cut with a metal punch attached to a vacuum pump.

Each well was filled with 7 µl of test and the corresponding standard serum for IgG, IgA and IgM (Combipack, Berhrringwerke AG, Marburg, FRG) using a Hamilton microsyringe (Hamilton Co., Reno, U.S.A.). The glass plates were then placed in humid boxes and incubated at 4°C for 18 h. Two diameter measurements of the rings formed at right angles to each other were made using a Hyland viewer with a micrometer eyepiece (Fisher Scientific Co, Pittsburgh, U.S.A.). The three respective immunoglobulin standard solutions were set up for each plate, together with two pooled normal human sera (NHS) to control plate-to-plate variation. Mean diameter readings of the NHS remained constant for all plates. Analysis for each immunoglobulin was performed on all samples at one time in order to avoid day-to-day variability.

Statistical analysis

Data for each group were summarized as means with standard deviations. The means of the five groups were compared using the one-way analysis of variance technique. Comparisons between any two groups were made using *t*-tests, using a pooled standard deviation from analysis of variance tables.

Results

The means and standard deviations of IgG concentrations obtained at all stages of gestation, and in age-matched controls of non-pregnant women, are shown in Table 1. Table 2 is the analysis of variance table simultaneously comparing the means of the five groups. The statistical analysis indicates that the means of the non-pregnant group showed a decrease in

Table 1. Serum immunoglobulin G concentrations of non-pregnant, pregnant and puerperal women

Groups	*n*	Mean ± standard deviation (mg/100 ml)
Non-pregnant	37	1719.73 ± 437.98
First trimester	19	1533.68 ± 466.04
Second trimester	48	1274.38 ± 301.34
Third trimester	33	1111.11 ± 219.57

Table 2. Analysis of variance simultaneously comparing the mean of five groups

Source of variation	Degrees of freedom	Sum of squares	Mean square	Variance ratio
Between group	4	4129130.00	2282282.00	18.79
Within groups	150	18094610.00	120630.73	
Total	154	27223740.00		

1st trimester *vs* controls	$t = 1.92$	$0.1 > P > 0.05$
1st *vs* 2nd trimester	$t = 2.61$	$0.01 > P > 0.05$
2nd *vs* 3rd trimester	$t = 2.58$	$P = 0.01$
3rd *vs* puerperium	$t = 0.14$	$P > 0.50$

IgG concentration when compared to that of the first trimester, but this was not significant ($t = 1.92$; $0.1 > P > 0.05$). This decrease was progressive throughout gestation (Fig. 1) from a maximum mean level of 1720 mg/100 ml in the non-pregnant women to 109 mg/100 ml in the third trimester. This decrease was found to be very significant between first and second trimesters ($t = 2.61$; $0.01 > P > 0.005$) and second and third trimesters ($t = 2.58$; $P = 0.01$). There was a marginal non-significant increase following delivery ($t = 0.14$; $P > 0.50$).

The mean values for IgA did not show any discernible trend (Fig. 1). There was, however, an elevation of mean IgA level following pregnancy (from 158 mg/100 ml to 178 mg/100 ml) and this was sustained until the second trimester; thereafter a decrease was noted.

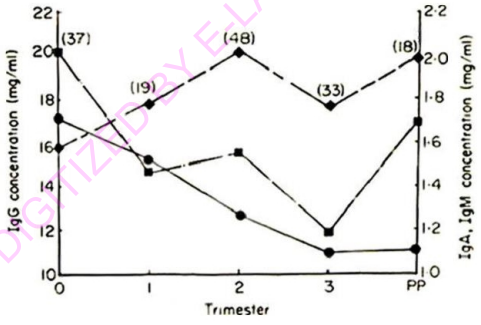

Fig. 1. The mean (maternal) serum immunoglobulin (●——●) IgG, (◆ - - - ◆) IgA, (■—·—■) IgM levels at each trimester of gestation. The numbers in parentheses indicate the number of investigations at each trimester of gestation. PP = post partum.

There was a slight increase from 177 mg/100 ml to 195 mg/100 ml post-partum (Fig. 1 and Table 3). Analysis of variance (Table 4) simultaneously comparing the means of the five groups did not reveal any significant differences ($P > 0.05$).

Like IgA, the mean IgM concentrations do not appear to show any definite trend (Fig. 1). The mean levels varied from 217 mg/100 ml in non-pregnant women to 119 mg/100 ml in the third trimester (Table 5). This value rose significantly to 171 mg/100 ml ($t = 2.75$; $0.01 > P > 0.005$) post-partum (Table 6). The results of statistical analysis also show significant differences between non-pregnant women and the first trimester groups ($t = 3.67$; $P < 0.001$) and between second and third trimester groups ($t = 2.96$; $0.005 > P > 0.001$) (Table 3).

Discussion

Theoretical considerations suggested for the progressive decline of IgG during pregnancy include: dilutional effects caused by hydraemia

Table 3. Serum immunoglobulin A concentrations of non-pregnant, pregnant and puerperal women

Groups	*n*	Mean ± standard deviation (mg/100 ml)
Non-pregnant	37	158.19 ± 50.66
First trimester	19	178.21 ± 64.85
Second trimester	48	211.75 ± 106.05
Third trimester	32	177.00 ± 79.99
Puerperium	17	195.18 ± 49.69

Table 4. Analysis of variance simultaneously comparing the mean of five groups

Source of variation	Degrees of freedom	Sum of squares	Mean square	Variance ratio
Between groups	4	65585.80	16396.45	2.59
Within groups	148	961382.30	6495.83	
Total	152	1026968.10		

of pregnancy, altered metabolic regulatory mechanisms, effect of elevated steroid hormones on protein metabolism during pregnancy and transplacental transfer to the foetus. Persuasive data supports a decrease of IgG during pregnancy [5,8,15–18]. Our results are consistent with these findings and we believe that all the above considerations may contribute to this decrease.

Table 5. Serum immunoglobulin M concentrations of non-pregnant, pregnant and puerperal women

Groups	*n*	Mean ± standard deviation (mg/100 ml)
Non-pregnant	30	217.00 ± 82.04
First trimester	19	147.05 ± 76.91
Second trimester	48	158.31 ± 66.71
Third trimester	32	119.03 ± 42.67
Puerperium	17	171.41 ± 73.64

However, there are still discrepancies in reports of IgM and IgA changes during pregnancy. A number of investigators have failed to demonstrate any noteworthy changes in the serum level of IgM [7,8,17,19], others have reported decreases [18,20], while McFarlane *et al.* [6] noted a progressive increase until just before parturition. If dilutional effects do account for IgG decline during pregnancy, although this has been contested [17], it is conceivable that IgM, whose distribution is mostly intravascular, should also decrease comparably. It is therefore possible that the initial decrease noted here may be related to the dilutional effect, as well as to the modulation of the immune response by steroid hormones, especially at this stage of pregnancy.

Evidence has accrued which suggests that pregnant women possess antibodies to allotypically incompatible antigens of foetal origin, such as ABO, HLA and B-cell allo-antigens, as a result of cellular traffic between mother and

Table 6. Analysis of variance simultaneously comparing the mean of five groups

Source of variation	Degrees of freedom	Sum of squares	Mean square	Variance ratio
Between groups	4	156231.00	39057.75	8.06
Within groups	141	678364.30	4811.09	
Total	145	834595.30		

1st trimester *vs* controls	$t = 3.67$	$P < 0.001$
1st *vs* 2nd trimester	$t = 1.01$	$P > 0.05$
2nd *vs* 3rd trimester	$t = 2.96$	$0.005 > P > 0.001$
3rd *vs* puerperium	$t = 2.75$	$0.10 > P > 0.005$
Puerperium *vs* controls	$t = 2.14$	$0.05 > P > 0.025$

foetus. These antibodies are mainly of the IgM class. In addition, streptococcal, staphylococcal and *E. coli* infections induce predominantly IgM antibodies. Ogbimi *et al.* (unpublished data) recently isolated these organisms from a considerable number of pregnant women in Benin City, which may explain the elevated serum IgM concentrations noted in parturients in this study. These observations could be pertinent to the questions of whether, when, and to what extent the mother responds to foetal antigens during gestation [8]. Our studies, in agreement with others [17,19], have shown no discernible trend in serum IgA concentrations throughout gestation: there was an increase up to the second trimester and a slight decrease in the third trimester, this was followed by a marginal rise post-partum. The increase and decrease were not statistically significant. The relevance of the IgA in the pregnant woman is therefore not immediately obvious.

Concentrations of serum immunoglobulins in the tropics, especially in the African population are reported to be higher than their Caucasian counterparts [5,10,11]. Recently, serum immunoglobulin levels in Benin City were shown to be higher than those at Ibadan [21]. Our results confirm previous results, including those of Wemambu [21], except that we failed to demonstrate higher IgG values in women at their third trimester of gestation in Benin City than the Ibadan investigators [12,13].

The findings in this study and others emphasize the considerable variability in immunological responses between individual pregnant females, especially in antibody production. Whether these changes are due to genetic factors, environmental factors, kinetic changes, or are a reflection of the differences in sensitivity between investigators will continue to be questionable.

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