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Transplacental transfer of measles and total IgG

G. GONÇALVES[1,2]*, F. T. CUTTS[3], M. HILLS[3], H. REBELO-ANDRADE[4], F. A. TRIGO[5] AND H. BARROS[2]

[1] *Instituto Nacional de Saúde Dr Ricardo Jorge, Delegação no Porto, Largo 1º de Dezembro, 4000–404 Porto Codex, Portugal*
[2] *Serviço de Higiene e Epidemiologia da Faculdade de Medicina do Porto, Alameda Prof. Hernâni Monteiro, 4200 Porto, Portugal*
[3] *London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK*
[4] *Instituto Nacional de Saúde Dr Ricardo Jorge, Centro Nacional da Gripe, Av. Padre Cruz, 1699 Lisboa Codex, Portugal*
[5] *Laboratório de Imunologia, Faculdade de Medicina do Porto, Alameda Prof. Hernâni Monteiro, 4200 Porto, Portugal*

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SUMMARY

This study was conducted to evaluate factors affecting the levels of total IgG (tIgG) and measles specific IgG (mIgG) in mother and cord sera, and the efficiency of transplacental transport of tIgG and mIgG. The study was conducted in four hospitals in Oporto, Portugal, where 1539 women and their newborns were enrolled. Measles IgG levels were lower among vaccinated mothers and respective cord sera than among vaccinated counterparts. Cord mIgG was strongly correlated with maternal levels in both vaccinated and unvaccinated groups. Transplacental transport efficiency (TTE) of mIgG decreased with increasing maternal levels, although almost one third of the observed effect was due to measurement error. The TTE was not affected by vaccination status. Monitoring maternal measles antibody levels and maternal vaccination status could be useful to determine when the age for measles vaccination can be reduced.

INTRODUCTION

The optimum age for measles vaccination must balance the age specific risks of disease and age-specific immunity induced by vaccine. Both are influenced by the levels of transplacentally acquired maternal antibodies [1]. Identification of factors affecting the levels of measles antibody passively transferred to newborns is therefore important to monitor potential changes in age at vaccination in maturing immunization programmes [2].

Previous authors have suggested that the efficiency of transplacental transport of IgG is lower when maternal levels are very high, whether for total immunoglobulin G [3] or for specific antibodies to measles [4] or tetanus [5]. However, the effect of measurement errors and intrinsic within-subject variability were not assessed in their studies.

We conducted a study to evaluate the factors affecting the levels of total and measles-specific IgG in mother's and cord sera and the efficiency of transplacental transport, in Oporto, Portugal. Factors investigated included maternal vaccination status, age, parity and gestational age.

* Author for correspondence.

METHODS

Study design and enrolment

The study was conducted in the Obstetric services of four hospitals in the conurbation of Oporto, Portugal, in 1993–4. Approval for the study was obtained from the regional primary health care board and from the ethical committees of the hospitals involved. After obtaining written informed consent, mothers were interviewed and data were collected from clinical records. At birth, a sample of 10 ml of blood was collected from the cord and from mothers. Sera were separated and frozen at −20 °C until testing. Maternal vaccination status was ascertained in women born after 1967, for whom measles vaccine had been available in Portugal. Mothers born before 1968 were classified as unvaccinated.

Measles IgG antibodies (mIgG) were measured in the Virology Department of the National Institute of Health, Lisbon, using a commercial enzyme immunoassay (EIA) (14458 Measles Virus IgG EIA, Diagnostica, Merck). An in-house standard serum was calibrated against the 2nd International Standard 1990, Anti-Measles Antibody, 66/202 [6] and used to convert optical density values to milli International Units (mIU) [7]. During initial experiments to calibrate the ELISA assay, repeat measurements were done on sera from 24 staff members (four tests/serum). This allowed us to assess the reproducibility of the assay (see below). Total IgG (tIgG) was measured at the University of Oporto. A standard serum (N Protein Standard SY) was used [8, 9] to establish reference curves for tIgG levels by immunonephelometry using Behring Nephelometry Systems [10]. Results were expressed in milligrams per decilitre (mg/dl).

Data analysis and statistical methods

All statistical analyses were carried out using the statistical package STATA [11]. The logarithms of maternal and cord tIgG and mIgG levels and the cord/maternal concentration ratios (CMR) were used in the analysis. Means of logarithms and their confidence intervals (CI) were exponentiated to give geometric means.

To assess the effect of measurement error on the relationship between the cord and maternal values of measles IgG we used an external estimate of the variance of measurement error in measles IgG obtained from four replicate measurements in 24 subjects. To describe the method, let X refer to the log of the true maternal mIgG level, and x to its value measured with some error. Normally error would include both biological variation and measurement error, but since we measured antibody levels at a unique moment in the lives of the mother and her newborn, only measurement is relevant. Similarly let Y refer to the true log cord IgG level, and y to its value measured with some error. It can be shown that the slope of the regression of y on x is related to the slope of Y on X by the equation

$$\beta_{yx} = \beta_{YX}(1-\gamma),$$

where $\gamma = \sigma_e^2/\sigma_x^2$, σ_e^2 is the variance of the measurement error, and σ_x^2 is the variance of the observed log maternal mIgG levels. Note that if there is no measurement error ($\sigma_e^2 = 0$, $\gamma = 0$) then $\beta_{yx} = \beta_{YX}$, but if the measurement error is appreciable in comparison with the variability in the log maternal mIgG values, then the absolute value of β_{yx} will be less than that of β_{YX}. Measurement error does not cause a bias in the intercept of the line. To correct the observed value of β_{yx} for measurement error we used

$$\beta_{YX} = \beta_{yx}/(1-\gamma).$$

A similar method is used to correct the regression of CMR on the maternal level for measurement error. The value of $(Y-X)$ is the true log CMR and $(y-x)$ is its value measured with error. Assuming that the errors in the log maternal IgG and log cord IgG have the same variance, σ_e^2 it can be shown [12, 13] that β', the regression coefficient of $(Y-X)$ on X by the equation

$$\beta' = \beta(1-\gamma)-\gamma.$$

If there is no measurement error ($\sigma_e^2 = 0$, $\gamma = 0$) then $\beta = \beta'$, but if the measurement error is appreciable in comparison with the variability in the log maternal mIgG values, then the value of β' will be less than β, even when $\beta = 0$. Following Blomqvist [12] we invert the equation relating β' to β and use

$$\beta = (\beta'+\gamma)/(1-\gamma)$$

to estimate the true regression of $(Y-X)$ on X.

RESULTS

General characteristics of mothers

Table 1 shows the general characteristics of the mothers enrolled. The mean maternal age was 27·4 years (range 13–46). The mean gestational age of

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Table 1. *General characteristics of mothers enrolled in measles vaccination study*

	n	%
Measles vaccination status		
Vaccinated	79	5·1
Unknown	135	8·8
Unvaccinated	1325	86·1
Parity (previous live births)		
0	855	55·6
1	448	29·1
2	134	8·7
3	55	3·6
4+	36	2·3
Unknown	11	0·7
Education (years)		
13+	105	6·8
10–12	143	9·3
7–9	228	14·8
< 7	787	51·1
Unknown	276	18·0
Pre-eclampsia		
Yes	15	1·0
No	1524	99·0
Total	1539	100·0

Table 2. *Concentrations of tIgG and mIgG in maternal and cord samples*

	tIgG (mg/dl)	95% CI (mg/dl)	mIgG (mIU/ml)	95% CI (mIU/ml)
Maternal	939	927–950	1459	1393–1529
Cord	1207	1193–1221	2130	2031–2235
CMR	1·29	1·27–1·30	1·46	1·42–1·50

infants was 39·5 weeks. Vaccination records were traced for 480 (78%) of the 615 women born after 1967; only 79 (16·5%) had been vaccinated.

Levels of mIgG and tIgG in maternal and cord sera

For 45 mother-cord pairs, there was insufficient sera to measure tIgG. Eight mothers had levels of mIgG less than or equal to 77 mIU/ml (3 S.D. below the geometric mean) and were assumed to represent unvaccinated women who had never had contact with wild measles virus; they were excluded from further analysis. The geometric means of the maternal and cord levels and the cord maternal ratio, with confidence intervals, are shown in Table 2.

The level of tIgG in maternal sera was significantly lower ($P < 0{\cdot}0001$ in a paired t test) than in cord sera. Similarly, the level of mIgG in maternal sera was significantly lower ($P < 0{\cdot}0001$) than in cord sera. The CMR for tIgG was significantly lower ($P < 0{\cdot}0001$ in a paired t test) than for mIgG.

Factors affecting mIgG levels

The most important determinant of maternal or cord mIgG levels was vaccination status (Table 3). Levels were much lower ($P < 0{\cdot}0001$) among vaccinated mothers than among unvaccinated mothers, and babies of vaccinated mothers had lower cord mIgG levels than those born to unvaccinated women ($P < 0{\cdot}0001$). The CMR did not, however, differ significantly between the two groups ($P = 0{\cdot}67$).

The only other variable that was significantly related to maternal mIgG level was maternal education (mIgG increased with over 12 years education). There was no additional effect of maternal age on either maternal or cord mIgG, after taking account of vaccination status. Variables significantly associated with CMR on multivariable analysis were gestational age, parity (CMR was reduced at parities of four and more) and pre-eclampsia (increased CMR in the presence of pre-eclampsia). Although the cord mIgG level was significantly associated with gestational age, parity and pre-eclampsia, adding these variables to a model containing maternal mIgG level only explained an additional 1% of the variance (data not shown).

Relationship between cord and maternal measles IgG

For measles IgG there is a strong linear relationship between cord and maternal levels ($r = 0{\cdot}87$), and this can be used to predict mean cord antibody levels reasonably well from maternal levels. This is not the case for total IgG where the variation in cord levels is far less well explained by variation in maternal levels than for measles IgG (Fig. 1). The linear regression of log cord mIgG on log maternal mIgG gives the following prediction equation:

$$\log(\text{cord}) = 1{\cdot}1695 + 0{\cdot}8914 \log(\text{maternal}) \pm 2 \times 0{\cdot}4714,$$

where the $2 \times 0{\cdot}4714$ allows for 95% of the variability in individual log cord values for a given log maternal value. Figure 2 (top) shows the 95% confidence limits on the predicted geometric mean of the cord mIgG for a given value of the maternal mIgG; the bottom graph shows the 95% confidence limits on an individual predicted mIgG cord titre for a given maternal mIgG.

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Table 3. *Maternal and cord concentrations of mIgG by vaccination status*

	Vaccinated		Unvaccinated	
	($n = 79$)	95% CI	($n = 1317$)	95% CI
Maternal (mIU/ml)	730	602–884	1534	1460–1613
Cord (mIU/m)	1083	908–1291	2230	2119–2347
CMR	1·48	1·35–1·63	1·45	1·42–1·49

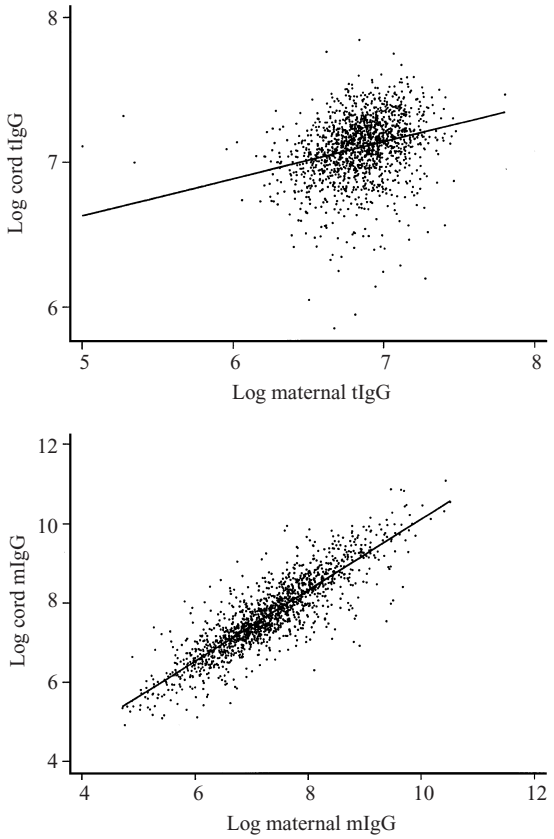

Fig. 1. Regression lines of log cord on log maternal levels of total IgG (top) and measles IgG (bottom).

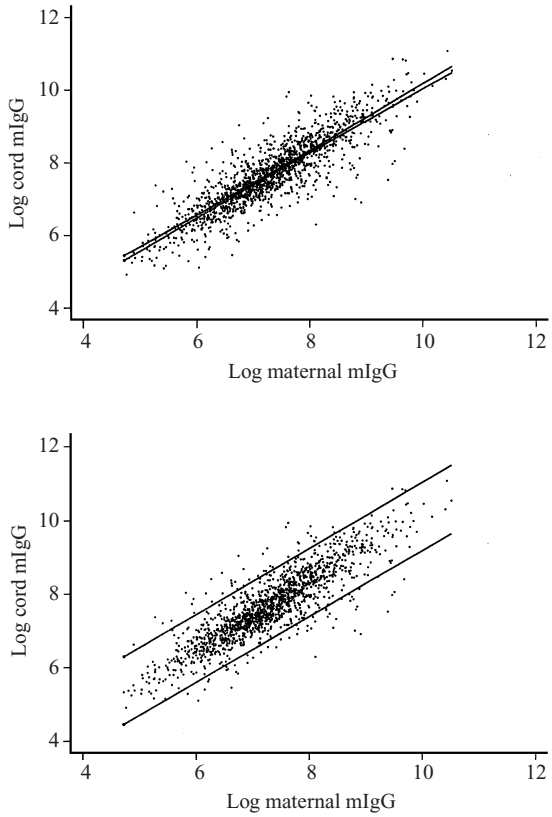

Fig. 2. The 95% confidence limits on the predicted mean of log measles IgG in cord sera for a given value of measles IgG in maternal serum (top graph). The 95% confidence limits on an individual predicted log measles IgG in cord serum for a given value of log measles IgG in maternal serum (bottom graph).

In each case, confidence limits were estimated in the usual way [14].

The slope of this relationship will be lower than it should be due to measurement error in the maternal level, and in order to take account of the effect of measurement error it is necessary to have some estimate of its variance. This was available for the measles IgG from the repeated observations of mIgG on 24 subjects. Each subject gave an estimate of this variance on 3 degrees of freedom (four repeat measurements), and the pooled estimate on $3 \times 24 = 72$ degrees of freedom was 0·0286 (95% CI 0·0222–0·0385).

Using this estimate of the variance of measurement error, the value of γ (see methods) is 0·03282, and the corrected slope is $0{\cdot}8914/(1-0{\cdot}03282) = 0{\cdot}9216$. The corrected prediction equation is:

$$\log (\text{cord}) = 1{\cdot}1695 + 0{\cdot}9216 \log (\text{maternal}) \pm 2 \times 0{\cdot}4714.$$

For example, the predicted log cord value when the maternal value for mIgG is 1500 mIU/ml is

$$\log (\text{cord}) = 1{\cdot}1695 + 0{\cdot}9216 \log (1500) \pm 2 \times 0{\cdot}4714$$

which is 7·9 (95% CI, 6·9669 to 8·8525), or 2723 (1061, 6992 mIU/ml).

The effect of measurement error on these results is small. If the uncorrected prediction equation is used,

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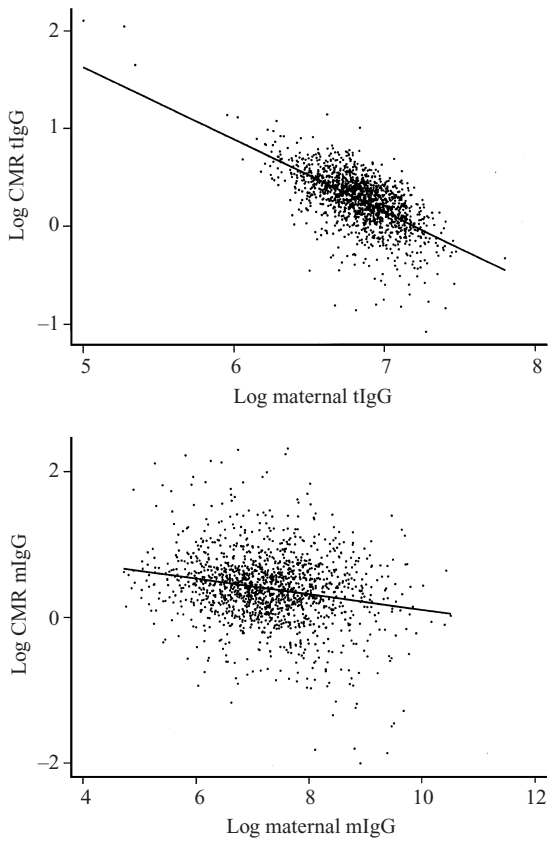

Fig. 3. Regression of log of cord/maternal concentration ratio of total IgG (top) and measles IgG (bottom) on corresponding log maternal levels.

for a maternal mIgG level of 1500 mIU/m, the predicted cord level would be 2183 (867, 5486) mIU/ml. For a maternal mIgG of 700 mIU/ml, the predicted cord level is 1107 (439, 2788) mIU/ml using the uncorrected regression and 1349 (535, 3398) mIU/ml using the corrected regression. These differences are unlikely to have clinical significance, as using either equation, the predicted time until babies can be effectively vaccinated (i.e. have a mIgG level less than 50 mIU [15]) is the same: six half lives in the first and five half lives in the second example.

Does the cord maternal ratio depend on the maternal level?

The plot of log cord maternal ratio versus log maternal antibody level is shown in Figure 3 (top graph for tIgG and bottom graph for mIgG). It appears that the CMR decreases with increasing maternal antibody levels for both measles and total IgG, but it is well known [13] that measurement error in maternal and cord IgG levels can invalidate such conclusions. Figure 3 (bottom graph) shows the apparent decrease in log CMR with increasing maternal mIgG level ($\beta' = -0{\cdot}1086$; $P < 0{\cdot}0001$). Using Blomqvist's method with $\gamma = 0{\cdot}0328$, and $1-\gamma = 0{\cdot}9672$, the corrected value of the regression of log CMR on log maternal mIgG is

$$\beta = (-0{\cdot}1086 + 0{\cdot}0328)/0{\cdot}9762 = -0{\cdot}0776.$$

Thus measurement error has accounted for 28% of the regression. To see whether what is left is significantly different from 0, we need the standard error for β which is 0·0143, not very different from the standard error of β', which is 0·0129. The value of β divided by its standard error is $-0{\cdot}0776/0{\cdot}0143 = 5{\cdot}4$ so there is still a highly significant dependence of log CMR on the log maternal value, for measles IgG, after allowing for measurement error.

DISCUSSION

The main determinant of the level of measles antibody in mothers and cord sera was maternal vaccination status, as previously shown [16–20]. The GMC of mIgG in maternal and cord sera of vaccinated women and their babies was about half that of unvaccinated women. This resulted from the lower maternal antibody levels in mothers rather than differences in CMRs. Other variables had only a small effect on maternal or cord antibody levels. Maternal education was associated with maternal serum levels of both mIgG and tIgG, but the association was in opposite directions: while higher educational levels were associated with high levels of mIgG, they were associated with lower levels of tIgG. There is no clear biological model to explain the reasons for this apparent contradiction. High parity (4+) was associated with reduced CMRs of mIgG and tIgG alike, and this was independent of maternal age. In countries where a higher proportion of women are of high parity, this may play a larger role in reducing cord antibody levels [21–23]. As expected, CMR increased with increasing gestational age [3]. The effect of pre-eclampsia on CMR of measles antibody is difficult to explain. The biological mechanisms of pre-eclampsia are not fully understood [24]. The number of women with pre-eclampsia was very small and the public health significance of the finding is low. Several other variables studied (including smoking during pregnancy) were not associated with any of the outcomes (data not shown). In some cases this confirmed previous work, e.g. infant's sex [16], birth weight [16, 17], and caesarean section [17], while the as-

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sociation of measles antibody level with maternal age reported by previous authors [17, 25, 26], appears to be due to confounding by vaccination status.

The CMR for mIgG observed in our study (1·46) was very close to that reported by Gendrel [4], but lower than those in other Caucasian populations [17, 27]. Different methods of measuring measles IgG levels were used in previous studies, but EIA gave very similar results for CMR to PRN assay in a small study we conducted [28], and in a study in the UK by Brugha and colleagues (personal communication, 1996). A Brazilian study has also shown good correlation between PRN and EIA in adults (high antibody levels) [29].

We have shown that the failure to take into account the potential effect of measurement error can bias the observed relationship between transplacental transport efficiency and maternal antibody levels. For measles, the apparent reduction in CMR at high maternal measles antibody levels [4] was reduced by 28% once the effect of measurement error was taken into account. For tIgG the CMR decreased as maternal levels increased, but in the absence of an estimate of measurement error for tIgG it was not possible to assess how much of this effect was due to measurement error. Our results are consistent with the hypothesis raised by Brambell, quoted by Gendrel [4], that although there is active transport of IgG across the placenta [30], the number of cellular receptors for IgG limit transplacental antibody transfer. Measles antibody appears to be transferred preferentially, with a stronger relationship between cord and maternal levels than exists for tIgG, and only a slight decrease in transplacental concentration efficiency across the range of maternal antibody levels observed in this study, as concluded by Lennon and Black [17, 27].

The main public health implications of the findings of this study concern the recommended age to vaccinate in countries where a high proportion of women have vaccine-induced immunity. Children born from vaccinated mothers have lower levels of measles IgG which will wane below those that interfere with vaccination earlier than in children born to unvaccinated mothers. In many countries, few women have documentation of the vaccines they received as children, and data on vaccination coverage of different age groups of women are imprecise. At the individual level, we found that the mother's antibody level did not predict the cord antibody level precisely. However at a population level, monitoring trends in mean measles antibody levels among samples of adult women (e.g. using sera from antenatal clinic attenders) could help programme managers to determine when to consider reducing the age for measles vaccination.

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