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Serum IgG subclasses levels in paediatric patients with pneumonia

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Abstract

Objectives: To determine the IgG subclass levels of patients admitted to Harare Central Hospital paediatric wards with pneumonia.
Design: A cross sectional study.
Setting: Harare Central Hospital, Departments of Immunology and Paediatrics, University of Zimbabwe; Department of Paediatric Immunology, University of Utrecht, The Netherlands.
Subjects: 56 paediatric patients.
Main Outcome Measures: IgG subclass profiles of children with pneumonia.
Results: Of the 56 children tested, 40 (71%) had antibodies to human immunodeficiency virus (HIV). The levels of IgG1 and IgG3 subclasses were significantly higher in HIV antibody positive children ($p<0.001$, $p<0.01$ respectively) than in those without detectable HIV antibodies in their sera. There was no significant relationship between IgG subclass levels and the presence of HIV p24 antigen. Furthermore, age and gender also had no significant influence on the levels of IgG subclasses in this population.
Conclusion: High levels of IgG1 and IgG3, but not IgG2 and IgG4, occur frequently in children with pneumonia and are associated with the presence of HIV antibodies.

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Introduction

Bacterial pneumonia is the commonest cause of morbidity and mortality in Zimbabwe.[1] The children most at risk are those infected with HIV.[2-4] We reported, in a recent study[2] that the great majority of children admitted to hospital with pneumonia have hypergammaglobulinaemia in immunoglobulins A (IgA), G (IgG) and M (IgM). IgG was the most raised immunoglobulin class with concentrations of up to 10 times that of normal age-matched reference ranges. The report was in agreement with other studies done previously.[3,5]

More recent studies[6,7] have shown that the concentrations of total IgG1 and IgG3, in various mucosal fluids, are increased in HIV-infected individuals while those of IgG2 and IgG4 are decreased. Furthermore, the concentrations of specific IgG1 and IgG3 antibodies to HIV virus are correspondingly increased in the same HIV-infected subjects while specific IgG2 and IgG4 antibodies were reduced. It is now established that IgG2 type antibodies are the most effective against encapsulated bacteria such as Streptococcus pneumoniae, particularly in young children.[8] It would appear that children with low IgG2 levels would have correspondingly low levels of IgG2-type antibodies

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and therefore a deficient humoral immune response to encapsulated bacteria. Such a deficient immune response would be further compromised by HIV infection.

The main objective of this study was to investigate the relationship between levels of IgG subclasses and the occurrence of pneumonia in children aged one month to six years with or without evidence of HIV infection.

Material and Methods

Patients.

The study population consisted of children, between the ages of one month and six years, admitted to hospital with pneumonia. The diagnosis of pneumonia was based on the World Health Organisation (WHO) clinical definition consisting of cough associated with difficulty in breathing.[9] Fifty six children with pneumonia, selected consecutively over a period of five months, were included in the study. Children with a history of asthma were excluded from the study. Parental consent was sort for all children used in the study.

Collection of Blood Samples.

Between two and five mls of venous blood was taken from each child into a plain tube for clotting. Serum was separated, aliquoted and stored at -20°C.

IgG Subclass Determinations.

IgG1, IgG2, IgG3 and IgG4 were measured by radial immunodiffusion (RID) assay using kits purchased from Binding Site (Birmingham, United Kingdom). All assays were performed according to the manufacturers' instructions. Briefly, equal volumes of serum from the patients were added to separate wells of the RID plates and the plates incubated at 37°C for 16 hours. Diameters of precipitin rings formed were measured with the aid of a magnifying glass and the concentrations calculated against the standards. Levels of these immunoglobulin subclasses were expressed in milligrams per millilitre (mg/ml). Normal reference ranges (+2SD) were taken from the publication entitled 'Handbook of Clinical Immunology Service'[10] used in routine Immunology Labs in Zimbabwe's Public Health Laboratories.

HIV Screening.

Serum from each patient was tested for HIV using three different enzyme-linked immunosorbent assay (ELISA) kits. All assays were performed in accordance with the manufacturers' instructions. Two independent HIV ELISA antibody kits (Genelavia, Sanofi Diagnostics, Pasteur, France; Human, Wiesbaden, Germany) were used to determine the presence of antibodies to HIV 1/2. Briefly, serum from each patient sample was incubated in HIV1/2 antigen-coated wells. After washing, an antibody-enzyme conjugate to human IgG was added and a second incubation done followed by further washing. A final incubation step with the enzyme substrate was done and colour development read at 405nm on the Coda Automated EIA Analyser (Biorad Laboratories, Hertfordshire, UK).

Because of persistence of maternal antibodies in children up to the age of about 18 months, a third HIV screening test, an HIV ELISA antigen test (Murex Chatillon, France), which detects the presence of the p24 antigen shed from the HIV 1 virus,[11] was used. Briefly, the method used was similar to the one above except that the ELISA plate wells were coated with antibody to the HIV virus

Results

Of the 56 patients studied, 40 (71%) had antibodies to HIV while 16 (29%) had no detectable antibodies to HIV. Of the 40 with antibodies to HIV, 22 had detectable HIV p24 antigen in their sera. HIV p24 antigen was not detected in any of the 16 HIV antibody negative patients. Although there was substantial variation between individuals in the levels of IgG subclasses, IgG1 subclass concentrations were markedly higher in HIV antibody positive (HIV Ab$^+$) than in HIV antibody negative (HIV Ab$^-$) patients (see Table I). Even when the medians for the IgG subclasses were compared between the HIV Ab+ and HIV Ab$^-$, as Table II shows, IgG1 and IgG3 median levels are significantly higher ($p<0.001$ for each of the 2 subclasses) in HIV Ab$^+$ patients. We found no significant differences in the levels of IgG2 and IgG4 between HIV Ab$^+$ and HIV Ab$^-$ patients.

Table I: A comparison of IgG subclass means between HIV Ab$^+$ and HIV Ab- children admitted to hospital with pneumonia.

	n	Mean (SD) IgG 1 mg/ml	Mean (SD) IgG 2 mg/ml	Mean (SD) IgG 3 mg/ml	Mean (SD) IgG 4 mg/ml
01-06 months					
HIV Ab$^+$	18	25.32 (15.33)	3.15 (1.55)	1.80 (0.87)	0.127 (0.12)
HIV Ab$^-$	3	9.64 (4.85)	2.24 (1.10)	1.11 (0.07)	0.15 (0.15)
07-12 months					
HIV Ab$^+$	5	47.1 (19.80)	4.45 (2.6)	1.41 (0.53)	0.35 (0.26)
HIV Ab$^-$	4	4.08 (1.34)	1.92 (1.41)	0.62 (0.13)	0.30 (0.31)
13-18 months					
HIV Ab$^+$	6	20.18 (14.12)	1.82 (1.35)	0.95 (0.96)	0.16 (0.14)
HIV Ab$^-$	3	3.86 (0.93)	1.50 (1.02	0.53 (0.10)	0.21 (0.16)
>18 months					
HIV Ab$^+$	11	27.07 (15.65)	2.13 (0.60)	2.28 (1.31)	0.22 (0.27)
HIV Ab$^-$	6	6.15 (1.27)	1.43 (0.63)	0.78 (0.41)	0.21 (0.11)

Reference Ranges:[10]

IgG subclasses

Age:	*IgGI (mg/ml*	*IgG2(mg/ml)*	*IgG3 (mg/ml)*	*IgG4 (mg/ml)*
6 months	*1.5-3.0*	*0.3-0.5*	*0.1-0.6*	*<0.1*
2 years	*2.3-5.8*	*0.3-2.9*	*0.1-0.8*	*<0.8*

Table II: A comparison of IgG subclass medians between HIV Ab$^+$ and HIV Ab$^-$ children admitted to hospital with pneumonia.

	HIV Ab$^+$ group (n=40) Median (Q1-Q3) mg/ml	HIV Ab$^-$ group (n=16) Median (Q1-Q3) mg/ml	p-value
IgG1	24.85 (16.45-34.05)	5.48 (4.1-6.1)	<0.001
IgG2	2.35 (1.69-3.56)	1.122 (0.78-2.6)	0.024
IgG3	1.71 (1.20-2.09)	0.67 (0.46-1.12)	<0.001
IgG4	0.13 (0.03-0.25)	0.15 (0.09-0.34)	0.24

IgG subclass levels were also compared between HIV p24 antigen positive ($HIVAg^+$) patients and those without detectable p24 antigen in their sera ($HIVAg^-$), in the same population group. Of the 56 patients, 22 (39%) were HIV Ag^+ while 34 (61%) were $HIVAg^-$. No significant differences were observed in the mean concentrations of each subclass between the two groups, as Table III shows. Similarly, no significant differences were observed when the medians of the IgG subclasses were compared between HIV Ag^+ and HIV Ag^- patients (Table IV).

Table III: A comparison of IgG subclass means between $HIVAg^+$ and HIV Ag^- children admitted to hospital with pneumonia

	n	Mean (SD) IgG 1 mg/ml	Mean (SD) IgG 2 mg/ml	Mean (SD IgG 3 mg/ml	Mean (SD) IgG 4 mg/ml
01-06 months					
HIVAg+	11	22.35 (15.11)	2.95 (1.19)	1.69 (0.50)	0.16 (0.13)
HIVAg-	10	23.88 (15.53)	3.09 (1.83)	1.71 (0.09)	0.12(0.13)
07-12 months					
$HIVAg^+$	0	—	—	—	—
$HIVAg^-$	89	29.98	3.33(2.50)	1.05 (0.56)	0.32(0.29)
13-18 months					
HIV Ag+	3	20.12 (15.11)	1.48(0.61)	0.35 (0.32)	0.17(0.11)
HIV Ag-	6	12.05 (12.36)	1.83(1.46)	1.04 (0.88)	0.18 (0.16)
<18 months					
HIV Ag+	8	25.03 (7.99)	2.27(0.59)	2.33(1.53)	0.16(0.12)
HIVAg-	9	14.97(19.66)	1.54(0.59)	1.23 (0.74)	0.26(0.29)

Reference Ranges:[10]
IgG subclasses

Age:	*IgG1(mg/ml)*	*IgG2 (mg/ml)*	*IgG3(mg/ml)*	*IgG4(mg/ml)*
6 months	*1.5-3.0*	*0.3-0.5*	*0.1-0.6*	*<0.1*
2 years	*2.3-5.8*	*0.3-2.9*	*0.1-0.8*	*<0.8*

Table IV: A comparison of IgG subclass medians between HIV Ag^+ and HIV Ag^- children admitted to hospital with pneumonia.

	HIV Ab^+ group (n=40) Median (Q1-Q3) mg/ml	HIV Ab^- group (n=16) Median (Q1-Q3) mg/ml	p-value
IgG1	20.15 (16.30-28.20)	8.43 (5.12-32.4)	0.18
IgG2	2.41 (1.69-3.15)	1.92 (1.15-3.49)	0.39
IgG3	1.57 (1.20-2.00)	1.16 (0.46-2.01)	0.13
IgG4	1.15 (0.03-0.23)	0.13 (0.06-0.31	0.77

The results were then subjected to multiple regression analysis with respect to age, gender, HIV Ab status and HIV Ag status. The coefficients of variation calculated across the IgG subclasses (see Table V) clearly showed that abnormally high levels of IgG1 and IgG3 in the patients studied were significantly associated with the presence of antibodies to HIV (p-values of <0.001 and 0.010, respectively). Age, gender and the presence of HIV p24 antigen had no significant influence on the levels of the IgG subclasses in this population.

Table V: Regression modelling of IgG subclass levels in children with pneumonia.

	Constant	Age	Gender	HIV Ab	HIV Ag	p value
IgG1	11.67	-0.03	-7.82	24.22*	-9.14	<0.001
IgG2	2.14	-0.02	0.66	-0.66	0.34	0.094
IgG3	1.84	0.01	0.44	-0.93*	0.01	0.010
IgG4	-0.11	0.00	0.12	0.04	0.05	0.283

**** Indicates significant predictors in the model.***

Discussion

The present study has established the pattern of distribution of IgG subclasses in children admitted to Harare Hospital with pneumonia, with or without evidence of HIV co-infection. Abnormally high levels IgG1 and IgG3 were significantly associated with the presence of HIV antibodies. The association between the levels of IgG2 and the presence of HIV antibody was not significant. Similarly, we did not find any association between the levels of IgG4 and the presence of HIV antibodies. To our knowledge, this is the first study of its kind to be conducted in Zimbabwe and the results are in agreement with other recently published reports.[6-7]

Factors such as age, gender and the presence or absence of HIV-p24 antigen did not appear to influence the pattern of IgG subclass levels in our study population. The apparent lack of association between levels of IgG subclasses and presence or absence of HIVp-24 antigen may be due to lower sensitivities of the p-24 assays[12] compared to those of established HIV antibody assays.

HIV antibody tests are the most widely used in adult subjects to screen for HIV. Such methods are not conclusive in children under the age of 18 months, because of persistence of maternally acquired antibodies.[13-14] Furthermore, HIV-p24 antigen tests have their limitations too as high sensitivities of about 80% appear to be achievable only in infants of up to two months — beyond that age the sensitivities tend to progressively diminish although specificity remains high.[12] Our results clearly suggest that abnormally raised IgG1 and IgG3 in children presenting with pneumonia appear to be strongly suggestive of concurrent HIV infection. Used as a diagnostic tool, IgG1 profiles would be relatively cheaper, particularly in the developing world, compared to other conventional techniques.

A recent study supports the same view.[15] Although the presence of abnormally high levels of IgG1 and IgG3 will not conclusively prove that a child is infected with HIV, but taken together with the mother's HIV antibody status could strengthen the clinician's suspicion that there might be underlying HIV infection. Appropriate clinical management regimes could then be applied to those children at risk found to have high IgG1 and IgG3 levels.

A future study could look at profiles of neutralising antibody isotypes in children born to HIV seropositive mothers in a longitudinal study. Special focus would be

given to those children who remain or become seronegative with time.

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