Performance of IgG avidity in an area endemic for schistosomiasis in Egypt

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فعالية رَغابة avidity الأيج ج IgG في المناطق الموطونة بالبلهارسيا في مصر

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الخلاصة: تم تقييم فعالية رَغابة الأيج G، في تشخيص العدوى الحادة أو المزمنة أو المتكرِّرة بالبلهارسيا، وتقييم شفاء المرضى المعالجين بالبرازيكوانتيل. درست مستويات الغلوبولينات المناعية في 111 من المرضى من بين 483 مصابا بالبلهارسيا المنسونية وافقوا على الانخراط في هذه الدراسة، وكان هناك 28 مريضاً من هؤلاء المرضى الـ 111 ممن وافقوا على الانخراط في الدراسة وهم جزء من أصل 40 مريضاً من بين مجموع المرضى المدروسين 483 الذين شفوا شفاء جزئيا (إذ لم يستجيبوا للجرعة الأولى من المعالجة بالبرازيكوانتيل ولكنهم شفوا تقريباً بعد الجرعة الثانية). وتم قياس كل من الأيج M والأيج G ومَنْسَب رَغابَة الأيج G، وتبيَّن أنه قبل المعالجة كان لدى جميع مرضى البلهارسيا ازدياد في مستويات الأيج G وكان لدى 75 ٪ منهم ازدياد في مستوى الأيج M. أما مَنْسَب الرَغابه فقد كان مرتفعاً بين جميع مجموعات الأعمار. إن ازدياد معدل الأيج M/الأيج G ومَنْسب الرَغابة لدى الأطفال المصابين بالبلهارسيا قبل المعالجة يدعم فكرة تكرر العدوى. وليس للمعالجة أي تأثير هام على المتغيرات المدروسة. وكانت الاستنتاجات أن اختبار رَغابة الأيج G لا يمكن استخدامه للتفريق بين العدوى المزمنة والعدوى الحديثة في المناطق الموطونة، وذلك بعكس مستويات أضداد الأيج M والأيج G.

ABSTRACT We assessed the performance of IgG avidity in the diagnosis of acute, chronic and recent (reinfection) on top of chronic schistosomal infections in patients treated with praziquantel. Immunoglobulin levels were studied in 111 patients with *Schistosoma mansoni* infection and 28 partially cured patients (not responding to the first dose of praziquantel treatment and almost cured after a second one). Before treatment all patients with schistosomiasis had elevated IgG levels, 75% of them also had increased IgM levels. Avidity index was high among all age groups. The increased IgM/IgG ratio and avidity index among children with schistosomiasis before treatment support the idea of reinfection. Treatment had no significant effect on the studied parameters. We conclude that unlike IgM and IgG antibody levels, IgG avidity test cannot be used to distinguish between recent and chronic infections.

Performance de l'avidité des IgG dans une zone d'endémie de la schistosomiase en Egypte
RESUME Nous avons évalué la performance de l'avidité des IgG dans le diagnostic de l'infection schistosomienne chronique et aiguë, ainsi que récente (réinfection) sur une infection schistosomienne chronique chez des patients traités au praziquantel. Les niveaux d'immunoglobulines ont été étudiés chez 111 patients atteints d'une infection par *Schistosoma mansoni* et 28 patients partiellement guéris (n'ayant pas répondu à la première dose du traitement par praziquantel et quasiment guéris après une deuxième dose). Avant le traitement, tous les patients atteints de schistosomiase avaient des niveaux élevés d'IgG et chez 75% d'entre eux il y avait également une augmentation des IgM. L'indice d'avidité était élevé dans tous les groupes d'âge. L'élévation du rapport IgM-IgG et de l'index d'avidité chez les enfants atteints de schistosomiase avant traitement tend à confirmer l'idée d'une réinfection. Le traitement n'avait pas d'effet significatif sur les paramètres étudiés. Nous concluons qu'à la différence des niveaux d'anticorps IgM et IgG, le test d'avidité des IgG ne permet pas d'établir la distinction entre les infections récentes et chroniques.

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Introduction

Schistosome immunity is a delicately balanced relationship between the immunological response of the host and the circumvention of this response by the parasite. Specific immunity appears to result from the interaction of antigens with mononuclear cells that circulate in blood and lymph. Two different types of lymphoid cells (B and T cells) mediate humoral and cellular immunity [*1*].

B lymphocytes are responsible for humoral immunity and antibody production. IgM antibodies are characteristic of a host response to many acute infections and IgG to chronic ones [*2*]. Researchers have reported that schistosome-specific antibodies can differentiate patients in acute phase of the infection from those of chronic phase. Higher IgM and lower IgG antibody levels have been seen in the acute phase while lower IgM and higher IgG in the chronic phase [*3*].

Antibody avidity means the strength of interaction of an antibody with the multivalent antigen. Depending upon the strength of this binding, the complex formed may or may not be dissociated. Antibody avidity is low after primary antigenic challenge and matures with time; it usually involves IgG antibodies [*4*]

Recently, an assay measuring this antigen binding avidity of IgG antibodies has been developed to separate the low-avidity antibodies produced at an early stage of infection from those with higher avidity that reflects past infection. This IgG avidity test has shown its value in schistosomiasis [*5*] and fascioliasis [*6*]. It has been reported that it may help in assessment of cure; low avidity may be found in successful treatment and high avidity in resistance to treatment [*7,8*].

We assessed the performance of IgG avidity in the diagnosis of acute, chronic, recent (reinfection) on top of chronic infections and in the assessment of cure in patients treated with praziquantel (PZQ) living in endemic areas.

Methods

The study was performed within the framework of a large epidemiological survey conducted in Abis 4 village, 12 km to the east of Alexandria (LM Abou-Basha, unpublished report, 2000). Assuming that the prevalence of *Schistosoma mansoni* in Abis area is 20% (LM Abou-Basha, unpublished report, 1984), in order to estimate the true prevalence of schistosomiasis infection, a maximum error of ± 0.5% in our estimate was considered acceptable. Therefore, the calculated sample size was 3000 cases.

Morning stool specimens were collected from each participant. The number of eggs per gram of faeces was determined by averaging the egg counts on three modified Kato thick smears (41.7 mg each) [*9*]. The participants were given a clinical examination and anti-schistosomal (PZQ) and anti-parasitic drugs were administered to infected villagers.

It is generally agreed that acute cases of schistosomiasis cannot be diagnosed clinically [*10*]. Therefore, blood samples were collected from 111 patients who consented to participate in the serological study (out of 483 villagers with a positive stool analysis for *S. mansoni*). PZQ (Biltricid) tablets were then given to the 483 patients positive for *S. mansoni* infection in a single dose of 40 mg/kg body weight. Two months after treatment the cure rate was 91.1%. The remaining positive patients

were given a second dose of PZQ. Of these, 40 had responded to the first drug dose and had reduced egg counts in stool and were therefore considered partially cured patients. Two months after the second dose, the overall cure rate was 98.7%, indicating 7.6% partial cure and 1.3% as response failure to PZQ treatment. After consent, blood samples were collected from 28 (70%) of the partially cured patients for serological study.

Serological studies

The immunoglobulin levels in schistosomiasis patients were studied before and after PZQ administration. Specific serum IgM and IgG were measured and compared with a control group of healthy individuals. The serum was separated from the collected blood and stored at –20 °C until used. Enzyme-linked immunosorbent assay (ELISA) was performed according to the method of Voller et al. [*11*] with some modifications. Lyophilized *S. mansoni* egg antigen (Teodor Bilhaz Institute, Cairo, Egypt) was used. It has been reported that this is more sensitive and specific than adult worm antigens irrespective of degree of purity [*3*]. Chequerboard titrations were performed to find the optimal dilutions for the antigen, test serum and conjugate to be used in the indirect ELISA.

Assay plates were sensitized with soluble egg antigen diluted in $NaHCO_3$/Na_2CO_3 buffer pH 9.6 for 2 hours at 37 °C and stored until used. Phosphate buffered saline/Tween (PBS/Tween) was used for all plate washings. Remaining free sites were blocked by adding 200 µL of 2% bovine serum albumin(BSA)/PBS. Negative, quality control and test sera diluted in 2% BSA in PBS/Tween were added in 100 µL volumes per well.

Conjugate (goat anti-human IgM and IgG peroxidase labelled) (Zymed) and substrate (O-phenylene-diamine (OPD)/H_2O_2) were added to the wells in 100 µL and 200 µL volumes respectively. The reactions were stopped by adding 25 µL of $2NH_2SO_4$. Absorbance at 490 nm was measured by an auto-ELISA reader. Optimal conditions were found to be 5 µg/mL, 1/150 and 1:5000 as regards the antigen, serum dilution and conjugate dilution respectively.

The adult reference value for IgM (95% confidence interval) is 0.171–0.300, while children's reference value for IgM is 0.204–0.270. Adult reference value for IgG (95% confidence interval) is 0.171–0.271, while children's is 0.181–0.251. Test results were considered positive if the optical density (OD) was equal to or greater than the upper confidence interval (mean ± 1.96 standard error of the mean) OD of the reference control sera (cut-off value).

To measure the avidity of specific IgG, the test was repeated after adding urea to the washing buffer. Urea acts as a hydrogen-bond disrupting agent and results in dissociation of low-avidity antibodies, whereas high-avidity antibodies remain antigen bound [*12*]. After performance of a pilot study using 6M and 8M urea, 6M urea gave the clearest separation and was accordingly used in the present work.

After measuring schistosomiasis-specific avidity using the "bind and break" method an avidity index (AI) [*13*] was calculated as follows

$$AI = \frac{\text{Absorption after urea wash}}{\text{Absorption after phosphate buffer wash}} \times 100$$

A low index indicates low avidity while a high index denotes high avidity.

Epi-Info was used for data entry and editing and statistical analysis was performed with SPSS (version 9).

Results

The study included 111 patients with schistosomiasis with a mean age ± standard deviation of 28.3 + 1.6 years and age range of 5–75years. There was no significant difference in the immunoglobulin levels and IgG avidity in relation to age, sex or intensity of infection, except that children had significantly higher IgM levels than adults. AI in all patients with schistosomiasis was high with a mean of 78.22 ± 2.00 in children and 75.91 ± 1.46 in adults ($P = 0.42$) (Table 1).

Before treatment, none of the patients with schistosomiasis had elevated IgM only. Normal IgM, elevated IgG and high AI were found in 33.3% of children and 39.1% of adults. Elevated IgM, IgG and high AI were found in 56.5% of children and 52.5% of adults.

There was no significant association between the immunoglobulin levels and AI regardless of treatment or age of villagers (Table 2).

The relation between IgM/IgG ratio and avidity index before treatment was studied. A significant negative correlation was observed among children ($r = -0.528$, $P < 0.05$) (Figure 1), while there was no significant correlation between these two parameters among adults ($r = 0.047$, $P = 0.69$).

Table 1 **IgM, IgG and IgG avidity index among villages of Abis 4 before treatment**

Variable	Optical density (nm)			IgG avidity index (%)
	IgM	IgG		
		Without urea	With urea	
Age (years)				
≤ 15 ($n = 29$)	0.47 ± 0.03	1.01 ± 0.04	0.80 ± 0.04	78.22 ± 2.00
>15 ($n = 82$)	0.40 ± 0.02	1.00 ± 0.02	0.78 ± 0.03	75.91 ± 1.46
P-value[a]	0.05	0.90	0.58	0.42
Sex				
Male ($n = 82$)	0.42 ± 0.02	1.01 ± 0.02	0.78 ± 0.02	76.49 ± 1.34
Female ($n = 29$)	0.42 ± 0.02	1.01 ± 0.04	0.79 ± 0.05	76.62 ± 2.57
P-value[a]	0.96	1.00	0.85	0.97
Intensity of infection (epg/stool)				
≤ 24 ($n = 33$)	0.41 ± 0.02	0.97 ± 0.04	0.74 ± 0.05	75.66 ± 2.71
24–96 ($n = 50$)	0.43 ± 0.03	1.01 ± 0.02	0.78 ± 0.03	76.06 ± 1.70
>96 ($n = 28$)	0.42 ± 0.03	1.05 ± 0.04	0.84 ± 0.04	79.51 ± 1.67
P-value[b]	0.83	0.27	0.26	0.35

[a] *P-value of student t-test.*
[b] *P-value of ANOVA test.*
epg = eggs per gram of stool.

Table 2 **Relation between IgG avidity index and immunoglobulin levels in children and adults infected with *Schistosoma mansoni***

Immunoglobulins	Children					Adults				
	No.	Avidity index[a]				No.	Avidity index[a]			
		Low		High			Low		High	
		No.	%	No.	%		No.	%	No.	%
Before treatment										
IgM normal and IgG elevated[b]	6	4	66.7	2	33.3	23	14	60.9	9	39.1
Elevated IgM and IgG	23	10	43.5	13	56.5	59	28	47.5	31	52.5
P-value			0.31					0.28		
After treatment										
IgM normal and IgG elevated[b]	2	1	50.0	1	50.0	6	5	83.3	1	16.7
Elevated IgM and IgG	5	1	20.0	4	80.0	15	7	46.7	8	53.3
P-value			0.43					0.13		

[a] *Avidity index was categorized into low or high according to median cut-off in each relevant group (children or adults before treatment/children or adults after treatment).*
[b] *The adult cut-off value for IgM was 0.300 and for children was 0.270. The adult cut-off value for IgG was 0.271 and for children was 0.251.*

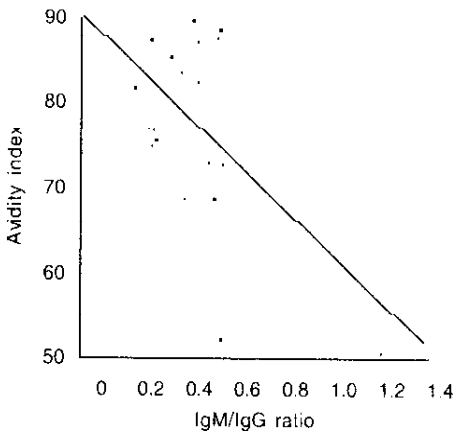

Figure 1 **Correlation between avidity index and IgM/IgG ratio in children before treatment**

Table 3 **Immunoglobulin values among 28 villagers after partial cure with praziquantel as compared to the control group**

Immunoglobulin[a]	Mean ± $s_{\bar{x}}$	Range	% elevation
Children (n = 7)			
IgM	0.47 ± 0.06	0.23–1.04	85.7
IgG	1.01 ± 0.08	0.28–1.26	100.0
Adults (n = 21)			
IgM	0.40 ± 0.03	0.15–0.97	71.4
IgG	1.00 ± 0.04	0.47–1.37	100.0
All villagers (n = 28)			
IgM	0.42 ± 0.03	0.15–1.04	75.0
IgG	1.01 ± 0.04	0.28–1.37	100.0

[a] *The adult cut-off value for IgM was 0.300 and for children was 0.270. The adult cut-off value for IgG was 0.271 and for children was 0.251.*
$s_{\bar{x}}$ = *standard error of the mean.*

Table 4 **Specific immunoglobulins and IgG avidity index before and 2 months after praziquantel treatment in 28 partially-cured patients with schistomsomiasis in Abis 4 village**

Group	Optical density (nm)			IgG avidity index (%)
	IgM	IgG		
		Without urea	With urea	
Children (n = 7)				
Before treatment	0.47 ± 0.07	1.00 ± 0.07	0.83 ± 0.08	82.13 ± 2.30
After treatment	0.69 ± 0.21	0.94 ± 0.09	0.75 ± 0.08	78.97 ± 2.44
P-value	0.22	0.04	0.02	0.24
Adults (n = 21)				
Before treatment	0.48 ± 0.04	0.89 ± 0.03	0.64 ± 0.05	70.42 ± 3.76
After treatment	0.44 ± 0.04	0.87 ± 0.03	0.63 ± 0.04	71.69 ± 2.90
P-value	0.11	0.54	0.80	0.51

Values are given as mean ± standard error of the mean.

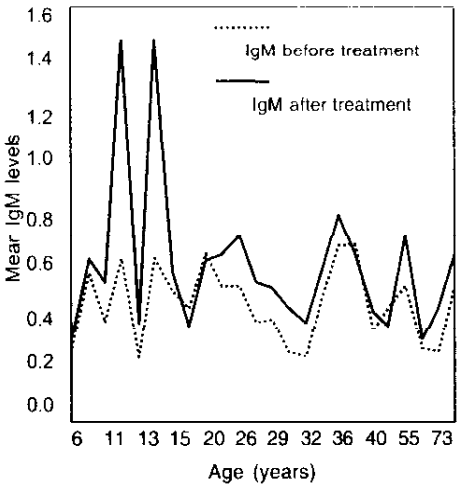

Figure 2 **Pattern of serum level of IgM in relation to treatment and age of villagers**

As regards antibody levels after treatment, about 75.0% of the examined cases had elevated IgM values, while 100% had elevated IgG values (Table 3). There was an increase in IgM levels in children and a decrease in adults after treatment but these changes were not significant. There was a significant decrease in IgG antibody levels 2 months after treatment in children but not in adults. Treatment had no significant effect on AI either in children or in adults (Table 4). There was a sharp rise in IgM levels after treatment among children (≤ 15 years) (Figure 2).

Discussion

There has been much debate about the technique of measuring humoral immune response in order to differentiate recent and chronic schistosomiasis and evaluate efficacy of treatment. Schistosomiasis-specific antigens have been reported to differentiate patients in the acute phase of infection from those in the chronic phase. Higher IgM/lower IgG antibody levels have been observed in the former and lower IgM/higher IgG levels in the latter [3]. Other investigators have reported high

levels of specific IgM and IgG in both acute and chronic schistosomiasis [7]. The authors suggested that IgM and IgG levels cannot be used to differentiate between clinical stages of the disease.

One of the suggested methods for differentiation is measuring the avidity of schistosomiasis-specific IgG antibodies. Furthermore, low-avidity antibodies may be helpful in assessment of cure to differentiate reinfected cases from those not responding to chemotherapy. Newly infected cases and those reinfected after successful treatment would show low-avidity IgG antibodies, while chronic patients and patients not responding to chemotherapy would have high-avidity IgG. IgG assay has been suggested to be a sensitive and specific measure for monitoring active treatment and active schistosomiasis infection. It may therefore be used to assess endemicity of the disease if low avidity IgG are sought [8].

We found that about 86% of the individuals examined had OD readings for IgM above the cut-off value. On the other hand, all participants with schistosomiasis cases had IgG readings above the cut-off value. This may indicate that individuals with schistosomiasis in Abis 4 village are either chronic or recent on top of chronic infection.

All participants with schistosomiasis had high AI with a mean of 75.9 ± 1.46 in adults and 78.22 ± 2.0 in children. These high levels suggest that all of the studied cases were in the chronic stage, which is in agreement with previous studies on infected villagers in Egypt that recorded a high mean AI of 71.4% for chronic schistosomiasis compared to a mean AI of 31.1% for recent infections [8]. High-avidity IgG has been previously reported in chronic experimental *S. mansoni* infection [7] and patients with chronic schistosomal infection [5].

We found that 33.3% of children and 39.1% of adults had normal IgM, elevated IgG levels and AI more than the median value of 78.4%. The normal levels of IgM in those with schistosomal infection could be explained by the findings reported elsewhere [14,15] that IgM antibodies appear 3 weeks post-infection, peak 7 weeks later and decline rapidly to a very low level. The elevated levels of both IgM and IgG and the high AI recorded in some participants in our study strengthened our belief that these cases had acute infection on top of chronic infection.

Moreover, the significant negative correlation between specific IgM/IgG ratios and IgG avidity before treatment in children could be explained by the fact that some children were in the acute stage of the disease. On the other hand, 56.5% of the children with elevated IgM values also had high IgG levels. In fact, elevated levels of IgG were found in all cases which indicates the chronicity of the infection while elevated IgM and IgG were present in 75.0% of the partially cured cases indicating acute on top of chronic infection.

These findings suggest that there is a continuous state of reinfection which makes it difficult to make a sharp distinction between the acute and chronic stages of schistosomiasis. Thus, AI is unlikely to be a reliable technique for the diagnosis of the clinical stages of schistosomiasis in endemic areas.

In endemic areas, the diagnosis of acute stage of schistosomiasis is difficult and several reports have confirmed the commonly held belief that acute forms of *S. mansoni* infection are seldom observed in endemic areas except among visitors to such areas [11]. Furthermore, most people

with chronic infection seen in endemic areas are asymptomatic; they lack marked hypersensitivity to *S. mansoni* infection and do not present with the acute clinical syndrome in the face of chronic re-exposure.

As regards the impact of treatment on immunoglobulins, there was no significant difference in the levels of all studied parameters before and 2 months after PZQ treatment of partially cured patients. This is consistent with previous reports [*15*].

The sharp rise of IgM in children after treatment can be attributed either to reinfection, as the magnitude of reinfection is higher in children [*16*], or due to the induction of an immunological response following chemotherapeutic treatment [*17*].

However, the fact that these children were still positive for *S. mansoni* after treatment disproves this last hypothesis and indicates that the sharp rise in IgM levels was mainly due to high reinfection rates. These results are consistent with previous studies that have demonstrated a wide range of antibody responses was positively correlated with subsequent reinfections and might therefore account for the continued susceptibility of younger children to reinfection [*18*].

As regards the aims of our study, the following are our most interesting findings.

- IgM and IgG antibodies are reliable in the diagnosis of acute and chronic infections in endemic areas.
- Although the IgG avidity test has proved to be useful in the diagnosis of acute infections in experimental schistosomiasis [*5–7*], it cannot be used to distinguish between recent and chronic infections in endemic ares because there is a continuous state of reinfection that makes it difficult to make a definitive distinction between acute and chronic stages.
- Stool examination was more reliable for detecting cases with partial cure than the studied immunological parameters (IgM, IgG and IgG avidity). The latter serological techniques showed no significant difference in the results of non-treated patients with schistosomiasis and those with partial cure in response to PZQ treatment.
- The sharp rise of IgM in children after PZQ treatment can be attributed to reinfection as the magnitude of reinfection is higher in children. We therefore emphasize the importance of good hygiene behaviour and ensuring access to chemotherapy to the vulnerable sectors of the population.

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