Specific IgG avidity in acute and chronic human fascioliasis

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النزوع المناعي النوعي للأضداد المناعية "ج" لداء المتورِّقات الكبدية في الإنسان

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خلاصة: إن اكتشاف النزوع المناعي للأضداد المناعية "ج" في الأمصال، أمر له فائدة محتملة في تشخيص العدوى الحادة والمزمنة. ولقد درسنا النزوع المناعي النوعي للأضداد المناعية "ج" في واحد وثلاثين مصاباً بداء المتورِّقات الكبدية، بهدف تقييم التطبيق السريري لهذا الاختبار من أجل تأكيد التشخيص في حالات الحضانة وللتفرقة بين الحالات الحادة والمزمنة. ومن بين واحدة وثلاثين حالة، كانت هناك 13 حالة حضانة، وكان بها مَنْسَب وسطي للنزوع المناعي قدره 57.28% ± 5.79%. وفي الحالات الثماني عشرة المزمنة كان مَنْسَب النزوع المناعي 68.80% ± 8.92%. وكان الفرق ذا دلالة إحصائية قوية. وخلاصة القول إن النزوع المناعي للأضداد المناعية "ج" وسيلة يُعتمد عليها في تحديد الطور الذي وصل إليه داء المتورِّقات الكبدية. ونقترح نقطة فَيْصلاً مقدارها 59.90% للتفرقة بين العدوى الحادة والمزمنة.

ABSTRACT The detection of IgG avidity in sera is potentially useful in the diagnosis of acute and chronic infection. We studied IgG avidity in 31 patients with fascioliasis, with the aim of evaluating the clinical application of this test to confirm the diagnosis of incubating cases and to distinguish between acute and chronic cases. Of the 31 cases, 13 were incubating and had a mean avidity index of 57.28 ± 5.79%. The 18 chronic cases had an avidity index of 68.80 ± 8.92%. The difference was highly significant. We conclude that IgG avidity is a reliable means of identifying the stage of fascioliasis and suggest a cut-off point of 59.90% to distinguish between acute and chronic infection.

Avidité spécifique des IgG dans la fasciolase chronique et aiguë chez l'homme

RESUME La détection d'une avidité des IgG dans les sérums est potentiellement utile pour le diagnostic de l'infection chronique et aiguë. Nous avons étudié l'avidité des IgG chez 31 patients atteints de fasciolase dans le but d'évaluer l'application clinique de ce test pour confirmer le diagnostic des cas en incubation et de distinguer entre les cas chroniques et aigus. Sur les 31 cas, 13 étaient en incubation et avaient un index d'avidité moyen de 57,28 ± 5,79%. Les 18 cas chroniques avaient un index d'avidité de 68,80 ± 8,92%. La différence était hautement significative. Nous concluons que l'avidité des IgG est un moyen fiable d'identifier le stade de la fasciolase et suggérons un seuil de 59,90% pour faire la distinction entre l'infection chronique et aigue.

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Introduction

Fascioliasis is recognized as an emerging human infection. The parasite infects a multiplicity of hosts in whom maturation and oviposition start at different times. In humans, the parasitic incubation (pre-patent) period has been estimated as 4 months [*1*]. During this period, the immature parasites affect the liver and form necrotic areas that end in fibrosis [*2*]. Early in infection, eosinophilia, high antibody titres and high circulating antigen values are the means of diagnosis [*3*]. With the appearance of ova in the stools, these values are significantly lowered [*4*].

Antibody avidity refers to the strength of interaction of an antibody with a 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, matures with time and it usually involves IgG antibodies [*5–7*].

Recently, an assay measuring the antigen-binding avidity of IgG antibodies has been developed to distinguish the low-affinity antibodies produced at an early stage of infection from those with a higher-binding affinity that reflects past immunity. This IgG avidity test has been valuable with many pathogens as both a front-line assay and as a means of distinguishing primary from secondary infections [*8–10*]. It is also helpful in assessing the time of the initial antigenic challenge. Diagnosis of the acute phase of fascioliasis is important, as treatment is effective during this stage and can prevent the harmful sequelae of the disease [*11*].

We aimed to study specific IgG avidity during pre- and post-patency in human fascioliasis. This may confirm the diagnosis early after infection, and it may also clarify the duration of infection.

Patients and methods

A total of 31 patients with single fascioliasis were studied (age range: 15–45 years), 13 of whom were incubating the infection and 18 were passing eggs in their stools. Patients in the acute stage were diagnosed clinically by pain in the right hypochondrium with fever, haematologically by leukocytosis with high eosinophilic count, and serologically by high specific antibody level together with negative stool examination [*11*]. Patients in the established phase (chronic stage) were diagnosed by the detection of ova in stools. Intensity of infection was established after examination of two thick-smear Kato slides of 41.70 mg each [*12*]. Sera were collected from all the patients and used in the indirect haemagglutination test (IHAT) (Fumouse Kit, France) and in the study of specific IgG avidity.

We used the Rivera Marrero et al. method for antigen preparation [*13*]. Live adult worms of *Fasciola gigantica* were placed in phosphate-buffered saline containing 0.8 mmol phenylmethylsulfonylfluoride for 3 hours at 37 °C. After incubation, the worms were removed and the medium containing the excretory-secretory (E/S) product was centrifuged. The supernatant was collected and its protein content determined [*14*]. This antigen has been found to be 100% specific for acute and chronic fascioliasis using IgM and IgG conjugates respectively [*15*].

To perform the IgG avidity enzyme-linked immunosorbent assay (ELISA) test, an enzyme immunoassay for determination of IgG antibody to *Fasciola* was carried out using E/S antigen according to the method described by Voller et al. [*16*]. To measure the avidity of specific IgG, the test was repeated after adding urea solution 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 [*17*]. A pilot study using 6 M and 8 M urea showed 6 M urea gave the clearest separation and thus was used in the present work. After measuring *Fasciola*-specific avidity using the "bind and break" method, an avidity index (AI) was calculated as follows:

$$\frac{\text{absorbance after urea wash}}{\text{absorbance after phosphate buffer}} \times 100$$

AI is an indicator of avidity. Therefore, a low index means low avidity while a high index denotes high avidity. To compare cases at the individual level, a cut-off point to differentiate between acute and chronic cases was suggested (after studying other possibilities) using the following formula:

$$\text{AI cut-off} = \text{mean AI in established cases} - 1 \text{ standard deviation}$$

Results

In the incubating group, the IHAT titres varied between 1/320 and 1/2560, while the group with established infection showed negative results, except for two with titres of 1/160 and 1/640 (Tables 1 and 2). Depending on the two Kato slides, the intensity of infection in established cases varied between 12 eggs/g of stool and 312 eggs/g of stool (Table 2).

Table 3 and Figure 1 show AI in acute and chronic cases of fascioliasis. In the incubating group, AI values ranged from 47.15% to 68.73% with a mean of 57.28 ± 5.787%. Patients with an established infection had AI values ranging from 54.48% to 91.36% with a mean of 68.80 ± 8.921%. The difference between the two groups was statistically significant ($P < 0.001$). The mean AI cut-off was 59.90%. Thus, values ≤ 59.90% denoted acute infection and those > 59.90% denoted chronic infec-

Table 1 Indirect haemagglutination titres and avidity index in patients with acute fascioliasis

Reciprocal indirect haemagglutination titres	Optical density values		Avidity index (%)
	Without urea	With urea	
2560	1.036	0.565	54.54
1280	0.889	0.518	58.27
1280	0.784	0.427	54.46
2560	0.644	0.331	51.40
640	0.324	0.187	57.72
640	0.789	0.372	47.15
2560	0.830	0.512	61.69
1280	0.971	0.583	60.04
1280	0.915	0.537	58.69
640	1.084	0.745	68.73
320	0.742	0.374	50.40
640	0.875	0.507	57.94
1280	0.698	0.444	63.61

Table 2 **Egg counts, indirect haemagglutination titres and avidity index in patients with chronic fascioliasis**

Mean egg count per g stool	Reciprocal indirect haemagglutination titres	Optical density values		Avidity index (%)
		Without urea	With urea	
216	–ve	1.021	0.667	65.33
60	–ve	0.965	0.685	71.00
72	–ve	0.961	0.878	91.36
60	–ve	0.597	0.358	60.00
72	–ve	0.401	0.268	66.83
132	–ve	0.498	0.316	63.45
48	–ve	0.676	0.539	79.73
60	–ve	0.570	0.402	70.53
24	160	0.782	0.426	54.48
12	–ve	0.493	0.312	63.29
96	–ve	0.325	0.214	65.85
12	–ve	0.726	0.445	61.29
84	–ve	0.941	0.625	66.42
312	640	0.692	0.467	67.49
96	–ve	0.359	0.229	63.79
60	–ve	0.536	0.449	83.46
108	–ve	0.719	0.494	68.71
24	–ve	1.026	0.776	75.63

Table 3 **Avidity index in acute and chronic fascioliasis**

Patients	Avidity index	
	Range (%)	Mean ± *s* (%)
Acute cases ($n = 13$)	4.15–66.73	57.26 ± 5.79
Chronic cases ($n = 18$)	54.48–91.36	68.80 ± 8.92

t = 4.07, P < 0.001.
s = standard deviation.

Table 4 **Accuracy of avidity index at the suggested cut-off point**

Avidity index (%)	True situation[a]	
	Acute cases	Chronic cases
≤ 59.9 (acute)	9	1
> 59.9 (chronic)	4	17
Total	13	18

[a] *According to stool examination and IHAT.*
Sensitivity to diagnose acute cases = 9/13 × 100 = 69.2%.
Sensitivity to diagnose chronic cases = 17/18 × 100 = 94.4%.
Overall accuracy = 26/31 × 100 = 83.9%.

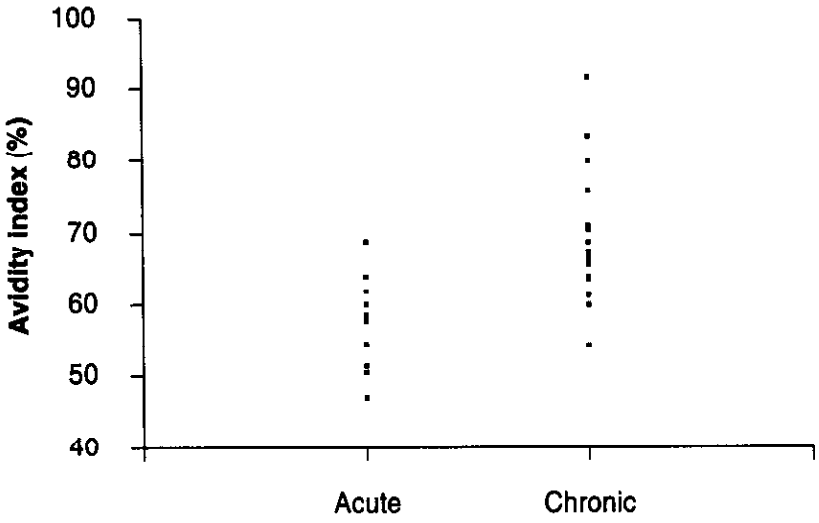

Figure 1 **Avidity index in acute and chronic cases of fascioliasis**

tion. Four patients in the acute phase had values above this point and one with established infection had a value below 59.90%. The validity of this cut-off point is examined in Table 4.

Discussion

We studied IgG avidity in human fascioliasis. It was measured as a function of hydrogen-bond dissociation using 6 M urea as the eluting agent, and AI was calculated for each case. A cut-off level was suggested to differentiate between acute and chronic cases. According to this level, the majority of cases in the acute phase had low avidity, while those in the chronic stage had high avidity. A previous study on experimental schistosomiasis reported low avidity antibodies up to the 10th week of infection, after which high avidity antibodies were detected [*19*].

IHAT is the reference standard for diagnosis of acute fascioliasis against which newer assays are compared [*20*]. However, 4 patients diagnosed by IHAT as having acute fascioliasis gave avidity figures above the cut-off point. It is known that *Fasciola* worms mature in 3–4 months in the human host, and maturation of the antibody response in other hosts has been reported to extend over a longer period of time [*21*]. The 4 patients were probably nearing the end of the incubation period, or they might be harbouring adult worms that were missed by stool examination. Thus, high avidity in a patient diagnosed as incubating fascioliasis points to the need for a revision of the diagnosis and for the performance of more stool examinations.

In the 18 patients with established infection, only 1 had low avidity, together with a positive IHAT. These two findings denote recent worm maturation, i.e. early chronicity. Another patient with chronic in-

fection had a positive IHAT, but had high avidity. This case could be explained by the relatively high infection intensity (312 eggs/g) with a high antibody level. Reinfection may offer another explanation to these findings.

Conclusion

IgG avidity ELISA, recently introduced for the serodiagnosis of some parasitic diseases, is useful in human fascioliasis. It confirmed the diagnosis of incubating infection and can thus be introduced as a screening test. Moreover, it distinguished incubating cases from established cases and thus could help determine the time at which infection began.

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References

1. Sampaio-Silva ML et al. Antigenic components of excretory–secretory products of adult *Fasciola hepatica* recognized in human infections. *American journal of tropical medicine and hygiene*, 1996, 54(2):146–8.
2. Abou Basha LM, Salem AI, Fadali GA. Ultrastructure of the liver before and after bithionol treatment. *Journal of the Egyptian Society of Parasitology*, 1990, 20:541–8.
3. Shehab AY et al. Detection of circulating E/S antigens in the sera of patients with fascioliasis by IELISA: a tool of serodiagnosis and assessment of cure. *Tropical medicine and international health*, 1999, 4(10):686–90.
4. Knobloch J. Human fascioliasis in Cajamarca/Peru. II. Humoral antibody response and antigenaemia. *Tropical medicine and parasitology*, 1985, 36(2):91–3.
5. Hedman K, Seppälä I. Recent rubella virus infection indicated by a low avidity of specific IgG. *Journal of clinical immunology*, 1988, 8(3):214–21.
6. Rousseau S, Hedman K. Rubella infection and reinfection distinguished by avidity of IgG. *Lancet*, 1988, 1(8594): 1108–9.
7. Enders G, Knotek F. Rubella IgG total antibody avidity and IgG subclass specific antibody avidity assay and their role in the differentiation between primary rubella and rubella reinfection. *Infection*, 1989, 17(4):218–26.
8. Hedman K et al. Recent primary toxoplasma infection indicated by a low avidity of specific IgG. *Journal of infectious diseases*, 1989, 159(4):736–40.
9. Hedman K, Vaheri A, Brummer-Korvenkontio M. Rapid diagnosis of hantavirus disease with an IgG-avidity assay. *Lancet*, 1991, 338(8779):1353–6.
10. El-Zayyat E et al. Evaluation of specific immunoglobulin G avidity enzyme-linked Immunosorbent assay (IgG avidity ELISA) in diagnosis of early and chronic schistosomiasis. *Journal of the Egyptian Society of Parasitology*, 1998, 28(3): 739–52.
11. Heshmat MG. Human fascioliasis in Behera province. *Egyptian journal of*

medical laboratory sciences, 1995, 1:55–61.

12. Katz NZ, Coelho PM, Pellegrino J. Evaluation of Kato's quantitative method through the recovery of *Schistosoma mansoni* eggs added to human feces. *Journal of parasitology*, 1970, 56(5): 1032–3.

13. Rivera Marrero CA, Santiago N, Hillyer GV. Evaluation of immunodiagnostic antigens in the excretory–secretory products of *Fasciola hepatica*. *Journal of parasitology*, 1988, 74(4):646–52.

14. Lowry OH et al. Protein measurement with the folin phenol reagent. *Journal of biological chemistry*, 1951, 193:265–75.

15. Shehab AY. *Detection of circulating Fasciola antigens (CFA) and of specific antibodies in the sera of infected patients. A chronological study* [Thesis]. Alexandria, Egypt, University of Alexandria, 1997.

16. Voller A, Bidwell DE, Bartlett A. The enzyme immunoassays in diagnostic medicine. *Bulletin of the World Health Organization*, 1976, 53(1):55–65.

17. Narita M et al. Immunoglobulin G avidity testing in serum and cerebrospinal fluid for analysis of measles virus infection. *Clinical and diagnostic laboratory immunology*, 1996, 3(2):211–5.

18. Thomas HI, Morgan-Capner P. The avidity of specific IgM detected in primary rubella and reinfection. *Epidemiology and infection*, 1990, 104(3):489–97.

19. Hassan MM et al. Low avidity antibodies in diagnosis of recent experimental schistosomal infection. *Journal of the Egyptian Society of Parasitology*, 1994, 24:193–8.

20. Farag HF, Youssef MY, Hassan ME. Anti-schistosoma and anti-*Fasciola* antibody levels in single and combined infections. *Journal of the Medical Research Institute*, 1988, 9:9–18.

21. Chen MG, Mott KE. Progress in assessment of morbidity due to *Fasciola hepatica* infection: a review of recent literature. *Tropical diseases bulletin*, 1990, 87:R1–R38.