EVALUATION OF HUMORAL IMMUNE RESPONSES ASSOCIATED WITH FASCIOLA INFECTION

Attallah A.M.[1], El-Shazely A.[2], Hammad K.H.[3], Saadh M.J.[1], And El-Waseef AM[4].

[1]R & D Dept., Biotechnology Research Center, New Damietta City, Egypt.
[2]Parasitology Dept., Faculty of Medicine, Mansoura Universty, Egypt
[3] Surgery Dept., University Students Hospital, Mansoura Universty, Egypt
[4]Chemistry Dept., Faculty of Science, Mansoura Universty, Egypt

(Received: 14 / 11 / 2007)

ABSTRACT

Recently, detection of Fasciola antibodies were used for the diagnosis of active infection in human. The present study aimed to evaluate the humoral immune responses associated with Fasciola Infection in human serum using specific antigen. Serum samples from 108 individuals with fascioliasis and 36 non-infected individuals were used. The *Fasciola gigantica* adult worm antigen preparation (FWAP) was used to evaluate humoral immune responses (IgM and IgG). Total anti- Fasciola IgM were detected in 91 (84.2%) of 108 serum samples, other 17 samples (15.8%) were negative for IgM. Total anti- Fasciola IgG were detected in 76 (70.3%) of 108 serum samples, other 32 samples (29.7%) were negative for IgG, both total anti-Fasciola IgM and total anti-Fasciola IgG were detected in serum of 100 cases (92.5%). The antibody was detected in serum using ELISA with degrees of sensitivity (84%), specificity (86%) for IgM. the ELISA assay sensitivity for IgG was (71%), specificity (83%). So, ELISA technique a simple and rapid immunoassay for detection antibodies during the prepatent period before the occurrence of damage to the liver.

Correspondence:

Dr. Abdelfattah M. Attallah-Director, Biotechnology Research Center-New Damietta-Egypt. Tel.: 020572402889, Fax: 020572401889, E-mail: amattallah@hotmail.com

INTRODUCTION

Human fascioliasis is a zoonotic disease caused by trematodes of the genus *Fasciola*, most commonly the two species, *Fasciola hepatica* and *Fasciola gigantica*. It has been considered an important veterinary disease. The World Health Organization now recognizes human fascioliasis as an important human parasitic disease [Mas-Coma, (1999)]. that occurs worldwide causing important economic losses to sheep and cattle, in commercial herds [Mauro et al., (2007)]. Human infection has been reported worldwide [Carrada-Bravo, (2003)]. It produces lesions of the liver and bile ducts. Infected animals are the main source for human fascioliasis, and sheep is the main reservoir for human contamination [El-Shazly et al., (2002)]. As many as 17 million people in the world, mostly children, are thought to be infected by *F. hepatica* (Hopkins, (1992)]. Humans are usually infected by the ingestion of aquatic plants that contain the infective metacercariae [Hillyer, (1998)]. Because the disease is worldwide in distribution and has varied clinical presentations. medical personnel should be aware of the possibility of infection in all geographic areas. A high index of suspicion requires establishing an early diagnosis and appropriate treatment [Rabin Saba, (2005)]. Diagnosis of fasciolosis is based on clinical findings and laboratory tests. The most reliable means is the finding of eggs in stool of infected individual despite the overwhelming consensus that this method is not wholly reliable [Hillyer, (1998)]. Serological diagnosis is preferred particularly since anti-Fasciola antibodies can be detected as early as 2 weeks post infection and can thus facilitate early chemotherapeutic intervention [Hillyer, (1998)]. The aim of the present study was the evaluation of humoral immune responses associated with Fasciola infection in human using *Fasciola gigantica* adult worm antigen preparation (FWAP). All individuals were diagnosed using parasitological methods of Fasciola eggs in stool.

MATERIALS AND METHODS

Study Subjects:

A total of 144 Egyptian individuals were included in the present study. They were 64 males and 80 females (age range: 4 to 65 years; mean 24 years). All individuals were diagnosed by parasitological methods for demonstrating Fasciola eggs in stool. they were 108

individuals showing Fasciola eggs in their feces. and the remaining 36 individuals were: 31 healthy individuals (No parasite infection as diagnosed by stool examination) and 5 samples from other parasite were used as negative controls (n=36).

Fasciola antigenic extract preparation.

Fasciola gigantica adult worm antigen preparation (FWAP) was prepared as described by [**Hillyer & Santiago de Weil (1977)**]. Briefly, adult *F. gigantica*, obtained from condemned bovine liver in local abattoir at Damietta, were washed repeatedly in cold 0.01 M phosphate-buffer saline (PBS; pH 7.4), over 1 h, to eliminate trace of bile and blood. The worms were then homogenized (2500 revolutions/ min for 20 min) in a glass homogenizer fitted with a Teflon pestle and held on ice; during the homogenization the pestle was slowly moved up and down to ensure all the worm tissue homogenized. The homogenate was centrifuged (7500 x g for 1h at 4° C). The protein content of a sample of the supernatant solution was then determined [**Lowry et al., (1951)**] before of the supernatant (FWAP) was split into aliquots and stored at –20 ′C until use.

Detection of IgG and IgM using ELISA.

After optimization of the reaction conditions, flat-bottomed, polystyrene. micro-titer plates (Corning Life Sciences, Acton, MA) were coated with the FWAP (2.5 µg/well in carbonate-bicarbonate buffer, pH 9.6). After blocking, 50 µl (per well) of 1:200-dilution, in PBS with 0.05% (v/v) Tween 20 (PBS-T20), of a human serum samples were added to each well. Serum from healthy human used as negative controls. The plates were incubated at 37°C for 2 h, washed. and then incubated, at 37°C for 1 h. with anti-human IgG or IgM alkaline phosphatase conjugate (The Binding Site. Bermingham, UK.) diluted 1:500 in 0.2% (wt/v) Bovine Serum Albumin in PBS-T20. After washing, the substrate was added and the plates incubated for 30 min at 37°C. Optical densities (O.D) were read at 490 nm using a micro-plate auto-reader (Metertech Inc. USA). The cut-off O.D for ELISA positivity was set as mean O.D plus three S.D. for the sera from healthy subjects.

RESULTS

Detection of anti-Fasciola IgM and anti-Fasciola IgG antibodies in Serum Using ELISA.

Serum samples from 108 individuals with fascioliasis were tested by ELISA for detection of human anti-Fasciola IgM and anti-Fasciola IgG antibodies. The anti-Fasciola IgM was detected in 91 (84.2%) serum samples of the 108 samples tested, while it was not detected in 17 (15.8%) serum samples **(Figure 1)**. The anti-Fasciola IgG was detected in 76 (70.3%) serum samples of the 108 samples tested, while it was not detected in 32 (29.7%) serum samples **(Figure 1)**. The positive cases for both total anti-Fasciola IgM and total anti-Fasciola IgG were detected in 100 (92.5%) of their serum samples, while they were not detected in 8 (7.5%) individuals showing eggs in their stool **as shown in Figure 2**. However anti-Fasciola IgM only was detected in 24 (22.2%) serum samples, anti-Fasciola IgG only was detected in 9 (8.3%) serum samples Both anti-Fasciola IgM and anti-Fasciola IgG positive in the same serum samples were detected in 67(62%) as shown in **Figure 3**. There was a significant correlation between optical density of IgG with IgM positive in the same serum samples (r = 0.734, p ⋍ 0.0.0001, n=67) **Figure 4**. The performance characteristics of ELISA as a rapid diagnostic assay of fascioliasis based on IgM and IgG detection in serum samples, revealed that sensitivity (84%; 71%) and specificity (86%; 83%) respectively among 36 controls. Positive predictive value (PPV) (95%; 93%), negative predictive value (NPV) (65%; 49%) and efficiency of the test was (85; 74%); respectively **(Tabel 1 & 2)**.
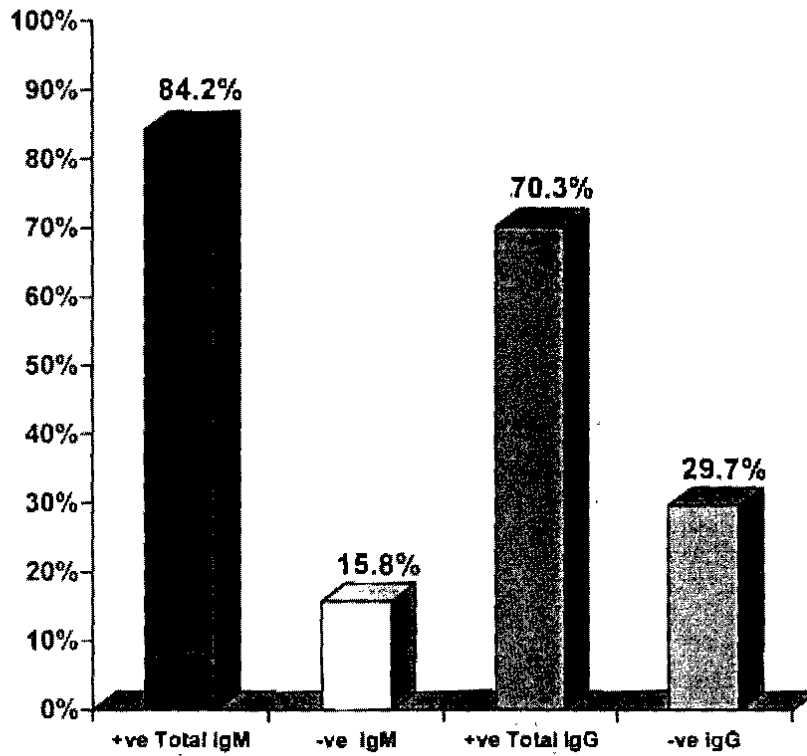
Fig. (1). Detection rate of anti-Fasciola IgM and anti-Fasciola IgG in 108 positive anti- Fasciola serum samples (patients with Fasciola eggs in stool).
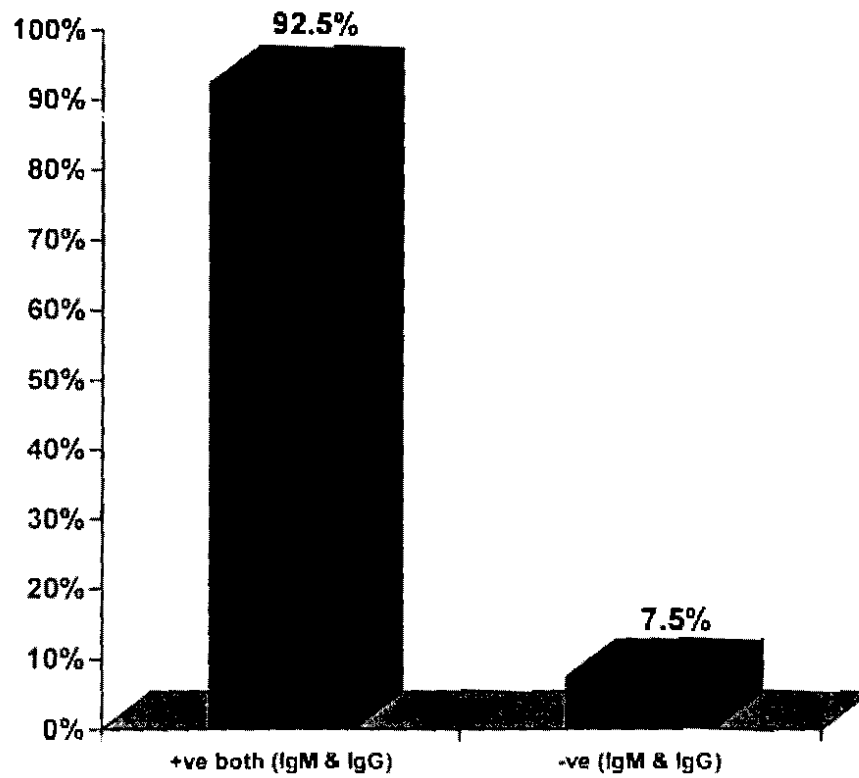
Fig. (2). Detection rate for both total anti-Fasciola IgM and total anti-Fasciola IgG in 108 fascioliasis sera (patients with Fasciola eggs in stool).
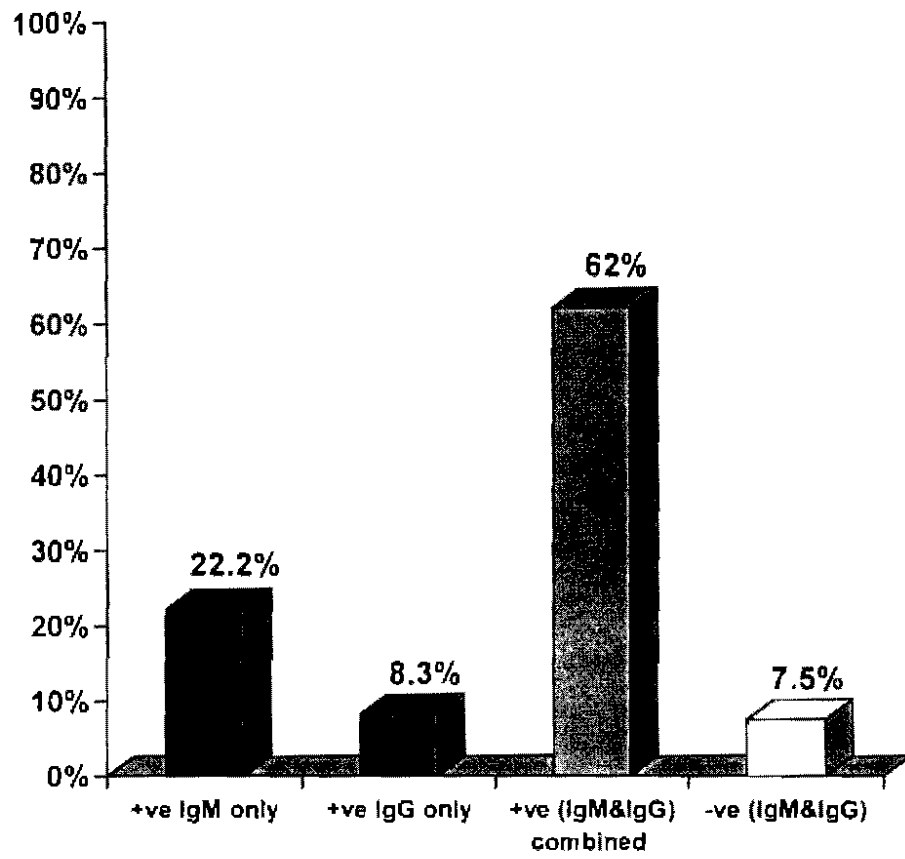
Fig. (3). Detection rate of anti-Fasciola IgM only, anti-Fasciola IgG only and (IgM & IgG) combined in the same sera of 108 infected patients sera (patients with Fasciola eggs in stool).
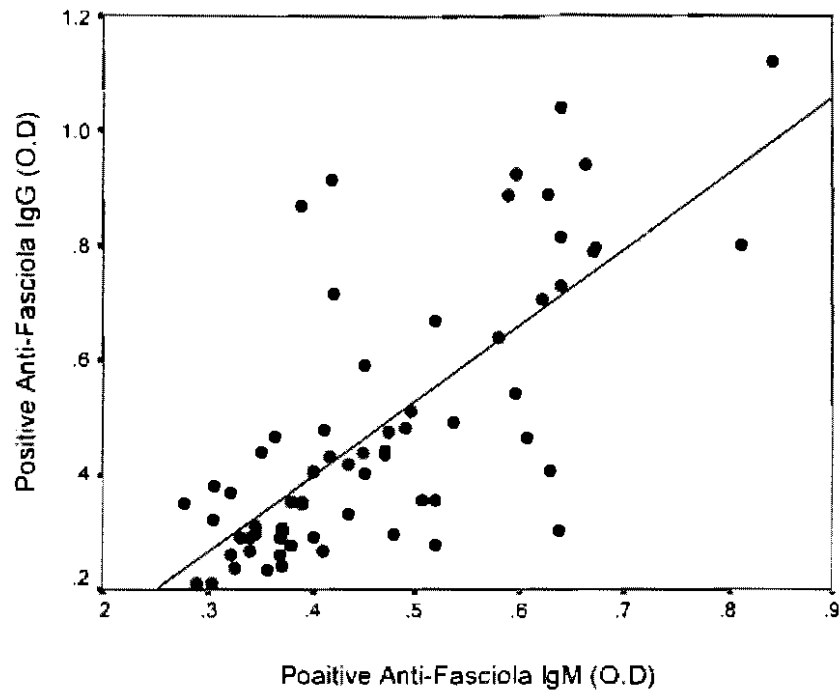
Fig. (4). Correlation between positive anti-Fasciola IgG and positive anti-Fasciola IgM in the same serum samples using ELISA (r = 0.734, p < 0.0001, n=67).

Table 1. The characteristics of ELISA for the detection of anti-Fasciola IgM antibody in comparison with Fasciola eggs in stool.

Stool analysis	Fasciola-IgM		Number
	+	-	
Positive	91(a)	17(c)	108
Negative	5(b)	31(d)	36
Total	96	48	144

Sensitivity = a / (a + c) x 100 = 91/ 108 x 100= **84%**
Specificity = d/ (b + d) x 100 = 31/ 36 x 100= **86%**
Efficiency = (a + d) / (a+b+c+d) x 100 = 122 / 144 x 100= **85%**
Positive predictive value = a/ (a + b) x100 = 91/ 96 x 100 = **95%**
Negative predictive value = d / (c + d) x 100 = 31/ 48 = **65%**

Table 2. The characteristics of ELISA for the detection of anti-Fasciola IgG antibody in comparison with Fasciola eggs in stool.

Stool analysis	Fasciola-IgG		Number
	+	-	
Positive	77(a)	31(c)	108
Negative	6(b)	30(d)	36
Total	83	61	144

Sensitivity = a / (a + c) x 100 = 77/ 108 x 100= **71%**
Specificity = d/ (b + d) x 100 = 30/ 36 x 100= **83%**
Efficiency = (a + d) / (a+b+c+d) x 100 = 107 / 144 x 100= **74%**
Positive predictive value = a/ (a + b) x100 = 77/ 83 x 100 = **93%**
Negative predictive value = d / (c + d) x 100 = 30/ 61 = **49%**

DISCUSSION

A high index of suspicion is required to establish a correct diagnosis. In areas of low endemicity, chronic cases are usually diagnosed coincidentally with cholecystectomy. At the least, patients with eosinophilia and abdominal pain should be evaluated for *F. hepatica* infestation. but physicians must remember that fasciola infestation can be present with a wide range of clinical syndromes. A history of ingestion of watercress or of other aquatic plants is important in the diagnosis, but a negative history cannot rule out the diagnosis **(Rabin Saba, 2005).** The definite diagnosis is confirmed only by demonstrating the parasite or its eggs. Clinical specimens include feces, bile, or duodenal fluid. Identifying parasite eggs in the feces is a simple diagnostic procedure. but has a low sensitivity because eggs do not appear during acute fascioliasis. Even in chronic forms, repeated stool examinations are often needed. and in many cases eggs are never detected **(Graham et al., 2001).** Measurement of humoral immune responses to Fasciola antigen. Serum samples from 108 individuals with fascioliasis were tested by ELISA for detection of human anti-Fasciola IgM and IgG antibodies. The anti-Fasciola IgM was detected in 91 (84.2%) serum samples of the 108 samples tested. while it was not detected in 17 (15.8%) serum samples. The anti-Fasciola IgG was detected in 76 (70.3%) serum samples of the 108 samples tested. while it was not detected in 32 (29.7%) serum samples. The combination of anti-Fasciola IgM and anti-Fasciola IgG

positive in the same samples were detected in 67(62%) while they were not detected in 8 (7.5%) serum samples. This lack of immune response in these patients may be due to immunodeficiency; only immune response to anti-Fasciola IgM was detected in 24 (22.2%) serum samples and only immune response to anti-Fasciola IgG was detected in 9 (8.3%) serum samples. There was a significant correlation between optical density of IgG with IgM positive in the same serum samples (r = 0.734, p < 0.0.0001, n=67). The high level of anti- Fasciola IgM and its biphasic pattern. High levels of anti- *F. hepntica* IgM have been previously described in rats **(Wedrychowicz & Turner, 1987; Poitou et al., 1993).** Commonly, the presence of specific IgM is said to indicate a recent infection. but a sequential release of antigenic products by *F. hepatica* could also explain the high level of IgM observed throughout the infection. Fasciola hepatica excretory secretory products (FhESP) specific IgM were produced from weeks post-primary infection (PPIW) 2 with peaks in PPIW 3 and 9-10; FhESP-specific IgG increased from PPIW 2 to 6 and became stable afterwards **(Chauvin, 1995).** Antibodies against antigens derived from adult fluke extracts or their excretory/secretory products (E/S) can be detected during the first 2–4 weeks after infection, providing a rapid and sensitive procedure for the diagnosis of the disease **(Santiago, 1988).** The antibody was detected in serum using ELISA with degrees of sensitivity (84%), specificity (86%) for IgM. the ELISA assay sensitivity for IgG was (71%) and specificity (83%). So, serological diagnosis is preferred particularly since anti-Fasciola antibodies can be detected as early as 2-4 weeks post infection during the prepatent period and can thus facilitate early chemotherapeutic intervention.

ACKNOWLEDGMENTS

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تقييم الاستجابة المناعية الخلطية المصاحبة للعدوى لطفيل الفاشيولا

عبد الفتاح عطا الله – عاطف الشاذلي – راضي حماد – محمد سعادة – أحمد الوصيف

الديدان الكبدية (الفاشيولا) هي أولى الديدان الطفيلية التي عرفها العلماء، فهي توجد في كل انحاء العالم و تنتشر بكثرة في المناطق التي تربى فيها الاغنام، وهي تصيب كلا من الانسان والحيوانات آكلات الاعشاب والاصابة بالفاشيولا تسبب خسائر اقتصادية كبيرة فـي الماشية. ويؤدي وجود الديدان باعداد كبيره الى انسداد القنوات الصفراويه، مما يتسبب فـي حدوث مرض اليرقان أو الصفراء وبعض الاضرابات الهضميه في أمعاء العائل.

يساعد التشخيص المبكر لهذا المرض في التخلص منه مبكرا وعلاجه ولقد اعتمـد الفحص المجهري لعينات البراز للكشف عن بويضات الفاشيولا والتي تظهر بعد 3-4 شهور من الاصابه أي في المرحلة المزمنة وبذلك يكون قد حدث تلف في أسجة الكبد وهذه الطريقه ذات حساسيه ضعيفه نتيجة لعدم انتظام توزيع البويضات في العينه واحيانا تكون غير حساسه وخاصه في الحالات الغير حاده كما أن البيض يفرز بشكل متقطع في المرحلة المزمنة.

لذلك تم تقييم الاستجابة المناعية للعدوى المصاحبة للفاشيولا من خلال تعيـين ال IgG والـ IgM بواسطة الاليزا وهي تظهر بعد 2-4 اسابيع من الاصابة وبـذلك نسـتطيع تشخيص المرض بشكل مبكر.