

Hepatic fibrosis due to fascioliasis and/or schistosomiasis in Abis 1 village, Egypt

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التليف الكبدي بسبب داء المتورقات أو داء البلهرسيات أو الداءين معاً في قرية أبيس "1"، جمهورية مصر العربية
ليلى محمود أبو باشا وعزيزة سالم وميرفت عثمان وصفاء الحفني وعادل زكي

خلاصة: أجريت دراسة وبائية حول داء المتورقات وداء البلهرسيات متفردين أو مجتمعين في قرية أبيس "1". قسم فحص عينات من 2492 شخصاً. وتبين أن داء المتورقات وحده أو مصحوباً بداء البلهرسيات كان أكثر انتشاراً بين الأطفال البالغين من العمر 5-15 سنة عنه بين الكبار. وتم تعيين مستويات الببتيد البروكولاجيني الثالث في المصل باعتبارها مؤشرات على التليف النشط. كما تم استعمال الباثولوجيا النسيجية وتخليط الصدى كمؤشرات على التليف المتوطد. ولوحظ أن مستويات الببتيد البروكولاجيني الثالث كانت أعلى في الأطفال عنها بين الكبار بدرجة ذات دلالة إحصائية، وفي العدوى المختلطة عنها في عدوى المتورقات وحدها. وقد وجد في الكبار، أن التليف حول الأورام الحبيبية، وكذلك التليف حول البواب من الدرجة الثالثة، كانا يصادفان في حالات العدوى المزوجة أكثر منهما في حالات العدوى المنفردة.

ABSTRACT An epidemiological study of fascioliasis and/or schistosomiasis was conducted in Abis 1 village. Stool specimens were collected from 2492 individuals and examined. Fascioliasis, alone or combined with schistosomiasis, was more prevalent among children aged between 6 years and 15 years than in adults. Serum procollagen III peptide (PIIIP) levels were determined as an indicator of active fibrosis, and liver histopathology and ultrasonography used as indicators of established fibrosis. PIIIP levels were significantly higher in children than in adults, and in mixed infections than in fascioliasis alone. In adults, fibrosis around granulomata detected by histopathology and grade 3 periportal fibrosis detected by sonography were encountered more frequently in dual than in single infections.

La fibrose hépatique due à la fasciolase et/ou la schistosomiase dans le village d'Abis 1 (Egypte)
RESUME Une étude épidémiologique de la fasciolase et/ou de la schistosomiase a été réalisée dans le village d'Abis 1. Des échantillons de selles de 2492 personnes ont été prélevés et examinés. La fasciolase, seule ou en association avec la schistosomiase, était davantage prévalente chez les enfants de 5 à 15 ans que chez les adultes. Les niveaux de peptide N-terminal du procollagène III (PIIIP) ont été déterminés comme indicateur d'une fibrose active, et l'histopathologie et l'ultrasonographie du foie utilisées comme indicateurs d'une fibrose établie. Les niveaux de PIIIP étaient considérablement plus élevés chez les enfants que chez les adultes, et dans les infections mixtes que dans la fasciolase seule. Chez les adultes, la fibrose entourant des granulomes détectée par histopathologie et la fibrose périportale au stade 3 détectée par sonographie étaient rencontrées plus fréquemment dans les infections doubles que dans les infections simples.

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Introduction

Schistosomiasis is the commonest disease in Egypt, with up to 70% of the rural population in endemic areas affected [1]. In Egypt, schistosomiasis is responsible for 70% of chronic liver disease among adults [2].

Human fascioliasis has recently been reported in several countries and has been identified as a cause of hepatic disorders [3]. In Egypt, the disease has been reported in several different governorates [4-9]. Combined infection of both schistosomiasis and fascioliasis is an increasing problem in rural communities [4]. Both parasites cause liver damage, inducing periportal fibrosis [10,11]. It has been reported that the degree of hepatic insult can be estimated from the amount of fibrous tissue, mainly collagen, formed [12]. In human fascioliasis, the most prominent histopathological findings are necrotic foci, degeneration of portal tracts and periportal fibrosis with deposition of collagen fibres inside the portal tract and ductal hyperplasia [10].

Although liver biopsy provides the best indication of the degree of liver damage, it does not reflect the dynamics of active fibrosis [10]. Recent studies have indicated that the process of fibrogenesis can be assessed from blood samples using sensitive immunological assays for specific components of connective tissue [13,14]. Most studies have focused on type III procollagen peptide, which is synthesized intracellularly by fibroblasts and transported into the extracellular space by the Golgi apparatus and microtubules, where it matures into collagen [15]. Assay of this peptide may be useful in disorders with ongoing fibrosis that eventually end in cirrhosis or fibrosis [16].

The magnitude of the problem of fascioliasis, alone or combined with schisto-

somiasis, and the impact on liver fibrosis in our community needs further attention. The aim of the present work was to quantify the prevalence of fascioliasis, alone or combined with schistosomiasis, in Abis 1 village, Egypt, and to clarify the importance of the connective tissue matrix marker procollagen III peptide (PIIIP), used together with standard sonographic and histopathological examination, as an indicator of active and/or established fibrosis.

Subjects and methods

Study area

The village of Abis 1, in an area where both schistosomiasis and fascioliasis are endemic, was chosen. Abis 1 is situated about 2 km east of Alexandria. A map was prepared and houses ($n = 403$) were numbered. The total study population comprised 3658 individuals, 2492 of whom (68.1%) provided stool samples for examination. Stool samples were collected twice from each individual on different occasions and examined in the laboratory using the Kato-Katz technique [17]. The prevalence of single and/or combined fascioliasis and schistosomiasis infections was determined.

Villagers with a positive diagnosis of schistosomiasis and/or fascioliasis were contacted. Those with a history of jaundice, viral hepatitis or intake of antirheumatic medication or schistosomicidal or fasciolocidal drugs within the preceding 6 months were excluded from the study. After explaining the objectives and benefits of the study and the need to be hospitalized for in-depth investigation and treatment, 281 eligible villagers agreed to participate in the study. Of these, 126 had *Schistosoma mansoni*, 111 *Fasciola* species and 44 had mixed infection with both parasites. All the participants were hospitalized, blood sam-

ples were collected for a complete blood picture, liver function tests [serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), serum alkaline phosphatase (SAP) and prothrombin activity] [18] were performed, and levels of hepatitis B surface antigen (HBsAg) [19] and the connective tissue matrix marker, PIIIP [20] were determined. Results were compared with those from a corresponding normal control of the same socioeconomic level, sex and age.

An ultrasonographic sheet was designed. Grading for periportal fibrosis was performed according to World Health Organization criteria: grade 1 = mild echogenic thickening (3–5 mm), grade 2 = moderate echogenic thickening (5–7 mm), grade 3 = marked echogenic thickening (> 7 mm) of the portal vein radical, scattered throughout the liver [21].

Histopathological study

Liver biopsies were taken after obtaining written informed consent from 43 patients representing all groups studied: 14 with *S. mansoni* infection (6 adults and 8 children), 14 with fascioliasis (6 adults and 8 children) and 15 with mixed infections (6 adults and 9 children). Biopsy was performed using an autovac large gauge (16) needle to take cores 2 cm long and 1 mm thick. Serial paraffin sections were taken and stained with haematoxylin and eosin, Mallory aniline blue stain for collagen, Van Gieson stain, and Gordon and Sweet silver stain for reticular fibres [22].

Sections were examined for:

- Liver parenchyma status, degree of fibrosis in portal tract, presence or absence of fibrosis;
- Detailed cellular description of granulomata: exudative (cellular), exudative productive (fibrocellular) or productive (fibrotic);

- Total number of granulomata in all cores with calculation of proportion of each type.

Statistical methods

Data were analysed using SPSS. The association between categorized variables was tested using the chi-squared test, the correlation coefficient was calculated for the linear relation between two continuous variables, and the *t*-test was used to compare values. The normality of the distribution of the continuous variables studied was also tested. The distributions of the variables (egg count, liver function and serum level of PIIIP) were all highly skewed, and non-parametric tests of significance were therefore applied whenever these variables were involved (Kruskal–Wallis test to compare means of more than two groups, the Mann–Whitney test for comparison of the means of two groups, and the Spearman rank test for correlations).

Results

In Abis 1 village, fascioliasis was more prevalent in children 5–14 years old than in adults. In children, single infection with *Fasciola* was found in 9.2% and dual infection with both *Fasciola* and *S. mansoni* in 4.1%, compared to 4.0% and 2.2% respectively in adults. There were significant differences between age groups in infection rate ($\chi^2 = 44.68$, $P = 0.0000$) (Table 1).

All the patients were negative for HBsAg. No significant difference ($P > 0.05$) in liver function tests (AST, ALT, SAP and prothrombin) was seen between groups in either children or adults. However, SAP levels were significantly higher in children than in adults ($P < 0.01$) (Table 2).

The highest levels of the connective tissue matrix marker PIIIP were observed in

Table 1 Prevalence of fascioliasis and schistosomiasis single or combined in Abis 1 by age group

Age group (years)	No. examined	Schistosomiasis		Fasciolosis		Dual infection	
		No.	%	No.	%	No.	%
<5	306	5	1.6	9	2.9	–	–
5–14	786	154	19.6	72	9.2	32	4.1
15–70	1400	318	22.7	56	4.0	31	2.2
Total	2492	477	19.1	137	5.5	63	2.5

$\chi^2_4 = 44.66, P = 0.0000.$

Table 2 Liver function tests of children and adults in different diagnostic groups

Test	Fascioliasis			Schistosomiasis			Dual Infection			P ^a
	No.	Mean	s	No.	Mean	s	No.	Mean	s	
AST (U/L)										
Children	61	35.7	24.03	42	6.0	15.03	23	27.5	10.18	> 0.05
Adults	50	29.5	19.35	84	29.6	14.55	21	30.1	10.48	> 0.05
P ^b	> 0.05			> 0.05			> 0.05			
ALT (U/L)										
Children	61	23.7	19.95	42	20.4	12.77	23	19.0	15.12	> 0.05
Adults	50	22.4	14.10	84	24.3	17.19	21	24.1	20.51	> 0.05
P ^b	> 0.05			> 0.05			> 0.05			
SAP (U/L)										
Children	61	19.8	7.11	42	20.4	7.41	23	22.8	8.34	> 0.05
Adults	50	11.9	8.20	84	12.4	7.00	21	12.3	6.19	> 0.05
P ^b	< 0.01			< 0.01			< 0.01			
Prothrombin activity										
Children	33	67.5	15.07	38	70.8	11.64	23	68.9	10.96	> 0.05
Adults	61	66.8	14.19	29	65.7	11.58	20	71.4	11.59	> 0.05
P ^b	> 0.05			> 0.05			> 0.05			

^aP: comparison between diagnostic groups.

^bP: comparison between children and adults in each diagnostic group.

AST = serum aspartate aminotransferase.

ALT = serum alanine aminotransferase.

SAP = serum alkaline phosphatase.

s = standard deviation.

Table 3 Procollagen III peptide (PIIIP) in children and adults in different diagnostic groups

Diagnostic group ^a	PIIIP (µg/L)									P ^b
	Fascioliasis			Schistosomiasis			Dual infection			
	No.	Mean	s	No.	Mean	s	No.	Mean	s	
Children	25	6.8	3.61	30	9.7	4.77	18	12.9	9.93	<0.01
Adults	20	2.4	2.11	62	8.4	7.90	11	6.7	3.36	<0.01
P ^c	<0.01			>0.05			0.05			

^aPIIIP was estimated for only 166 individuals.

^bP: comparison between diagnostic groups.

^cP: comparison between children and adults in each diagnostic group.

s = standard deviation.

those with dual infection, lower levels were detected in those with schistosomiasis and the lowest levels in those with fascioliasis (Table 3). In those with fascioliasis, alone or combined with schistosomiasis, age was inversely proportional to the logarithm of procollagen level ($P < 0.01$).

The bivariate intercorrelation between relevant variables (Table 4) showed that in those with fascioliasis, age was inversely correlated to the logarithm of *Fasciola* egg counts, PIIIP and SAP levels. In dual infection, age was inversely correlated with PIIIP and SAP levels (Table 4).

Histopathological results

Liver biopsies of children in all patient groups revealed hepatic cell vascular hypertrophy (increased cell volume) and granulomata in the exudative stage (cellular) with no fibrous element (Figure 1). The cellular reaction was more evident in cases with mixed infection.

In adults with schistosomiasis, of 30 examined granulomata (4–6 per biopsy), 13 (43.3%) were exudative, 13 (43.3%) were exudative productive and 4 (13.3%) were productive.

In adults with fascioliasis, liver cells showed vascular hypertrophy. The liver

Table 4 Bivariate correlation between age and log of *Fasciola* egg count, procollagen III peptide (PIIIP), serum alkaline phosphatase (SAP) and prothrombin activity in cases of fascioliasis alone or dual infection

Variable	Age	
	Fascioliasis alone	Dual infection
<i>log Fasciola egg count</i>		
n	108	44
r	-0.34	0.14
P	0.0	0.38
PIIIP		
n	45	29
r	-0.57	-0.514
P	0.0	0.004
SAP		
n	108	44
r	-0.54	-0.599
P	0.0	0.0
Prothrombin activity		
n	65	43
r	-0.24	0.11
P	0.051	0.49

was crossed by fluke tracks which were necrotic or in which collagen had been laid down, with cellular infiltration. Frequently (30% of cases), there were areas of com-

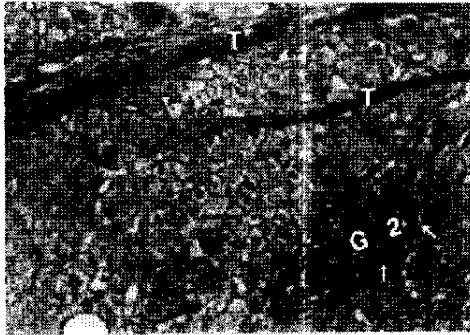


Figure 1 Section through liver tissue of child with fascioliasis, showing cellular granuloma, (G) and 2 larval tracks (T) containing collagenous fibres and cellular reaction (Van Gieson stain $\times 125$).



Figure 3 Section through liver section of adult male with mixed fascioliasis and schistosomiasis, showing prominent fatty change in liver and bile duct hyperplasia (?) (H&E $\times 125$).

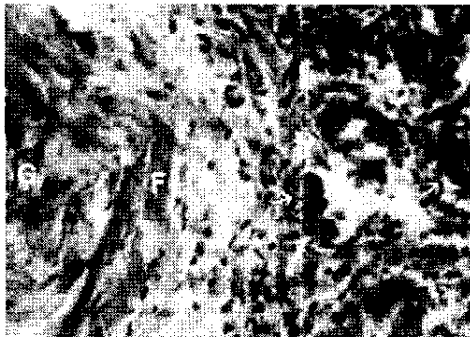


Figure 2 Section through liver tissue of an adult infected with fascioliasis, showing exudative productive granuloma (G) with cellular infiltration around the fibrous element (F). The portal tract shows bile duct hyperplasia (?) (H&E stain $\times 125$).

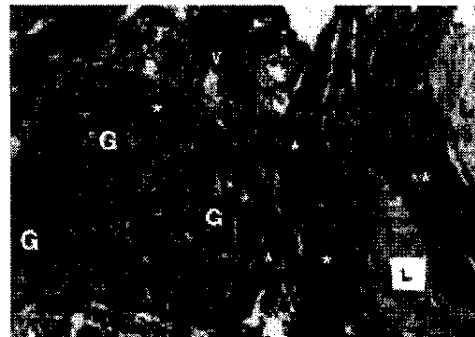


Figure 4 Productive hepatic granuloma (G) of the same patient as Figure 3 (mixed infection), with marked deposition of collagen around granulomata. Note sclerosis of hepatic artery (a) and portal vein (v) by the vascularized connective tissue (Van Gieson stain $\times 125$).

plete liver necrosis with loss of nuclei. Portal tracts were enlarged with abundant fibroblasts and proliferating ducts were seen (Figure 2). In these patients, of 28 examined granulomata (4–6 per biopsy), 15 (53.6%) were exudative and 13 (46.4%) were exudative productive; no productive granulomata were found.

In adults with mixed infection, the hepatocytes showed vascular hypertrophy and increased intracellular deposition of fat (Figure 3). There was marked deposition of fibres leading to sclerosis of the hepatic artery and portal vein by the vascularized connective tissue (Figure 4). Of 26 examined granulomata (4–5 granulomata per biopsy), 1 (3.8%) was exudative, 9 (34.6%) were exudative productive and 16 (61.5%) were productive ($\chi^2_4 = 34.78$, $P < 0.001$).

All adult patient groups showed a similar degree of deposition of collagen and reticular fibres around granulomata.

Ultrasonography

In patients with fascioliasis, ultrasonography of the upper abdomen showed fluke density in the gall bladder in 22.2% (12/54) of children aged 5–14 years compared to 8.1% (3/37) in adults. Grade III periportal fibrosis was detected in 11.3% (9/80) of individuals with *S. mansoni*, in none of the patients with *Fasciola* alone (0/37) and in 23.1% (6/26) of those with mixed infection.

Discussion

A relatively high prevalence of established fascioliasis (5.5%) was detected in the study area, previously known as an area where schistosomiasis was endemic (19% of the present study population). Dual infection with both parasites was detected in 2.5% of the villagers examined. These figures are comparable with those reported by Abou Basha [4] in a study of the prevalence

of fascioliasis and schistosomiasis in Abis 2 village, which found a prevalence rate of 5.1% established fascioliasis, 25.8% schistosomiasis and 1.6% dual infection.

Fascioliasis alone and fascioliasis combined with schistosomiasis among children in Abis 1 village is an important public health problem. This result confirms the previous reports of a greater prevalence of established fascioliasis among children (7.2%) [4]. In the present study, more than half the cases of fascioliasis diagnosed were in children. Furthermore, a greater intensity of infection was detected in children, as confirmed by the significant inverse relation between age and egg count. The logarithm of the *Fasciola* egg count correlated with SAP. The increased intensity of infection in children may be due to their more immature immune systems. The increased SAP level in children of all patient groups may reflect the fact that higher SAP levels are generally detected in children because of their rapid bone growth [23]. The results of the liver function tests (ALT, AST, prothrombin) confirm that these enzymes are not significantly affected except in late stages of infection.

The levels of PIIP were significantly increased in schistosomiasis and fascioliasis, and maximally in dual infections. This may be due to stimulation of fibroblast proliferation by both parasites, and subsequent procollagen synthesis. It has been reported that both schistosomiasis and fascioliasis induce fibroblast recruitment [10, 24]. The markedly higher level of procollagen in patients with dual infection compared with single infection with either parasite, and the absence of significant difference in liver function in any patient group, supports the value of estimating PIIP levels in such cases.

In our study, the increased vascular hypertrophy in children and adults may be attributed to the congestion and local oede-

ma following schistosomiasis [25] and/or fascioliasis. The intracellular deposition of fat within hepatic cells may be due to toxins released by both parasites, ischaemia of liver parenchyma following *S. mansoni* oviposition, ischaemia due to excessive fibrosis, or shrinkage of hepatic vasculature due to excessive deposition of fibrous tissue around the blood vessels.

The histopathological results of this study are in line with those of the ultrasonography but not with procollagen levels, most probably because the first two reflect chronic established fibrosis while the latter reflects ongoing active fibrosis.

Conclusion

In the Abis 1 area, fascioliasis is a significant public health problem. Children are at higher risk than adults, and the higher levels of PIIP detected in children could indi-

cate more active reversible fibrogenesis. Further study of the impact of early antihelminthic treatment on the progress of liver fibrosis in children is recommended.

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