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Autor: Favez, Gérard / Fiorelli, Gemino / Magnenat, Gérald
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Demonstration of Circulating Antibodies Directed against the Casoni Antigen during Echinococchiasis in Man Preliminary Communication

GÉRARD FAVEZ [1], GEMINO FIORELLI [2] and GÉRALD MAGNENAT [1]

Clinique Cevey-Sylvana, Department of Medicine (Head: Prof. A. Vannotti), University of Lausanne Medical School, 1066 Epalinges, Switzerland

Hypersensitivity reactions specific to corresponding antigen during echinococcchiasis in man are well established. Reliability of both the skin test (Casoni) and the complement fixation technique (Weinberg) are diversely appreciated, the former being usually considered as more sensitive than the latter, which in turn may be more specific than the former. Bentonite particles flocculation (GARABEDIAN et al. 1959; NORMAN et al. 1959; KAGAN et al. 1959) coated with scolex extract or cyst fluid, or hemagglutination, show a higher degree of specificity. Nevertheless scolex extract or cyst fluid are not easily available in countries where echinococchiasis is unfrequent but where the diagnosis of this disease is occasionally to be considered.

The present work was initiated to investigate the reliability of the Casoni antigen (easily obtained on the market) in hemagglutination. The passive hemagglutination method which has been employed in this study has provided fairly reliable results in tuberculosis (FAVEZ & HADORN 1963; FAVEZ et al. 1966; FAVEZ 1968).

Material and method [3]

Serum samples were obtained in Sardinia (Italy) from 18 patients and in Switzerland from 2 patients with surgically confirmed echinococchiasis in 18 cases; in one elderly patient with bilateral lung cysts, who was not operated, the Casoni skin test was strongly positive (both early and delayed reaction). The serum samples were kept in vacuum flasks provided with carbonic snow and forwarded to Switzerland. Serum samples were obtained from 10 healthy controls and one patient supposed to have an abdominal cyst.

A 2.5%-suspension of human red cells of group O Rh-, suspended in a 0.15-molar buffer solution pH 7.2 [4] was treated with tannic acid dilution 1 : 20,000 for 10 min at + 37°C, and was then centrifuged at 380 g. The sedimented erythrocytes were washed with the same buffer solution and with 0.9% NaCl solution. The tanned washed blood cells were again suspended in a 2.1%-concentration of a 0.15-molar buffer solution pH 6.4 [5] and 0.1 ml Casoni antigen [6] diluted 1 : 10,000

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[1] Department of Medicine (Head: Prof. A. Vannotti), University of Lausanne Medical School (Switzerland).

[2] Institute of Special Medical Pathology and Clinical Methodology (Head: Prof. N. Dioguardi), University of Cagliari Medical School (Italy).

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[4] 8.15 g NA_2HPO_4, 2.45 g KH_2PO_4, 4.5 g NaCl in 1,000 ml sterile distilled water.

[5] 3.44 g NA_2HPO_4, 6.92 g KH_2PO_4, 4.5 g NaCl in 1,000 ml sterile distilled water.

[6] The lyophilized Casoni antigen used in this study was manufactured by Behring-Werke Ltd., Marburg/Lahn, Germany.

in buffer solution pH 6.4 was added to each tube. 0.1 ml of 2.5% suspension of tanned and coated red cells was added to each tube. Centrifugation at 280 g was performed at + 4°C. The globular residue was washed in the buffer solution pH 7.2 and Coomb's human antiglobulin serum was added (0.05 ml diluted 1 : 10 per tube). The tubes were delicately shaken, allowed to rest, then centrifuged at 380 g at + 4°C. The degree of agglutination was estimated.

Serum samples with elevated titers were incubated overnight with 0.1 ml Casoni antigen diluted 1 : 10,000 and then tanned and coated red cells were added.

Tanned erythrocytes exposed to Casoni antigen were added to the supernatant removed after hemagglutination has been obtained.

Table 1. Locations of the hydatid cysts and anti-Casoni titers (20 cases).

Patients	Sex	Age	Date operation 1966	Date sampling 1966	Casoni		Blood eosinophils %	Titers (reciprocal)	Controls	Location of the echinococchiasis
					immediate	delayed				
Or. An.	F	28	22. 1.	22. 1.			9	640	0	unilocular cyst of the right lung
Sc. Ol.	F	14	10. 2.	24. 1.	neg.	neg.	6	640	0	unilocular cyst of the right lung
Pi. An.	M	73		13. 2.	+++	+++	8	160	0	bilateral lung cysts. Patient not operated
Ma. Ba.	F	25	20. 12. 1965	25. 1.				640	0	unilocular cyst of the left lung
Or. Ma.	M	18	24. 4.	24. 4.	+++			640	0	unilocular cyst of the right lung
Ba. Di.	F	16	24. 4.	21. 4.	++		1	640	0	unilocular cyst of the liver
Bo. Of.	F	49	20. 5.	19. 4.	neg.	neg.	3	640	10	cyst located under the right kidney. Proved to be of no hydatid origin
Pi. Er.	M	52	20. 5.	27. 4.				640	0	rupture into the peritoneal cavity of an unilocular cyst of the liver. Peritonitis
Mo. An.	M	23	7. 4.	27. 4.				1,280	0	traumatic rupture into the peritoneal cavity of an unilocular cyst of the liver
Pi. Ma.	F	14	26. 4.	27. 4.	+++		6	640	0	multiple cyst of the liver
Mu. Ce.	M	61	3. 1.	27. 4.				1,280	10	unilocular cyst of the liver
Sc. Ro.	M	19	19. 4.	19. 4.	neg.	neg.	5	640	10	unilocular cyst of the left lung
Ar. An.	M	20	18. 1.	18. 1.			0	640	0	unilocular cyst of the left lung
Pe. Lu.	M	49	23. 12. 1965	22. 1.			2	640	0	unilocular cyst of the liver
Po. Pa.	M	24	23. 1.	23. 1.				80	0	unilocular cyst of the liver
Ma. Vi.	F	20	4. 1.	4. 2.	+++	+++	9	1,280	0	multiple cysts of the liver
En. Di.	M	27	24. 1.	24. 1.	+++		6	640	0	unilocular cyst in the left lung (cyst in the right lung operated 8. 3. 1965)
Mu. An.	M	31	10. 1.	24. 5.	+++		4	1,280	10	unilocular cyst of the liver
Ar. Ro.	M	23	21. 1.	21. 1.	neg.	neg.	1	1,280	0	unilocular cyst of the left lung
Co. On.	M	34	22. 1.	22. 1.			10	640	0	multilocular cysts of the liver

Results

Hemagglutination titers in sera from patients with proved echinococchiasis (lung cysts 10 cases, liver cysts 9 cases) were 1 : 1,280 in 5 cases; 1 : 640 in 12 cases; 1 : 160 in one case and 1 : 80 in one case. A titer of 1 : 640 has been observed in a patient exhibiting at the operation a cyst located under the right kidney, but it proved to be not of hydatid nature (Table 1). In controls the titers were 1 : 10 or there was no hemagglutination at all.

No hemagglutination was observed in sera which were incubated overnight with Casoni antigen and thereafter addition of coated red cells. When coated erythrocytes were added to the supernatant removed after hemagglutination occurred, no further hemagglutination took place.

Discussion

The results obtained so far in this study suggest that the Casoni antigen is a suitable material for the hemagglutination test to be performed in echinococchiasis in man. Further more, in this small series at least, the method has proved to be reliable as titers have been obviously higher in patients as compared with controls; only one false positive hemagglutination occurred. According to both the inhibition test and the fractional exhaustion test, the demonstrated circulating antibodies may be specific of the antigenic determinants present in the Casoni material. The results suggest that the passive hemagglutination method using the commercial Casoni antigen instead of scolex extract or cyst fluid may be an adequate tool in the diagnosis of echinococchiasis in man. A comparison with the Casoni skin test is not possible in this study because it has been carried out only in 11 patients (4 negative and 7 positive reactions). Larger series will be investigated in order to evaluate the reliability of the method.

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