

HUMAN MUSCLE SARCOCYSTOSIS IN RELATION TO NON-SPECIFIC RHEUMATIC DISEASES AND RHEUMATOID ARTHRITIS

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ABSTRACT

Human muscle sarcocystosis is a parasitic infection acquired by ingestion of the sporocysts of the Sarcocystis species.

Materials & Methods: *This study was performed on 22 non-specific rheumatic patients, 21 rheumatoid arthritis (RA) patients and 10 apparently healthy persons as controls. Using the Sarcocystis fusiformis antigen (Ag.), serum samples of the patients and the controls were tested for the presence of Sarcocystis species antibodies using the Western Blot technique.*

Results: *Out of the 22 patients of the first group, 14 were positive (63.7); of the 21 patients of the second group five were positive (23.8 %). They detected several band ranges from 15-116 KD. Most of these samples had eosinophilia ranged from 7-20. Non of the control group serum reacted against Sarcocystis Ag. Statistically the difference between the two groups was significant ($P < 0.04$).*

Conclusion: *Sarcocystis infection may be an important cause of the non-specific rheumatic diseases associated with myositis.*

INTRODUCTION

Non-specific rheumatic manifestations that occur with undiagnosed cases are common. Some of these are associated with parasitic infections. Muscle sarcocystosis is a possible cause of idiopathic cardiac diseases, rheumatic complaints and myositis (*Habeeb et al., 1996*).

Human muscle sarcocystosis is a rarely diagnosed infestation caused by a coccidian parasite belonging to the genus *Sarcocystis* (Dubey et al. 1989). *Sarcocystis* infection in humans has 2 distinct forms. In the first form, ingestion of water or food contaminated with sporocysts from the faeces of a carnivore (eg, dog, wolf) is followed by sporozoite penetration of the intestinal wall. Proliferation in vascular endothelium and subsequent hematogenous dissemination leads to invasion of skeletal and cardiac muscles. These cysts subsequently disintegrate with accompanying vasculitis and fibrosis of the tissues (myositis).

The second form of Sarcocystosis in man occurs after ingestion of undercooked meat infected by *Sarcocystis* cysts. The trophozoites undergo sexual reproduction and maturation in the intestinal tract. Infective oocysts are shed into the stool. A systemic phase and a subsequent tissue phase do not occur in this form of the infection (Kiel, 2002).

Sarcocystosis has worldwide distribution. Most cases of human sarcocystosis occur in Southeast Asia. A study of autopsy specimens of patients in Southeast Asia showed a prevalence rate of 21% in 100 consecutive patients evaluated (Wong & Pathmanathan, 1992). The seroprevalence of Sarcocystosis in Malaysia was estimated at 19.8%.

In the United States, more than 60 cases of muscle involvement by the *Sarcocystis* species have been described, mostly in collections of case reports of 5-10 cases (Kiel, 2002). Seven members of 15-man U.S military team that had operated in rural Malaysians had Sarcosystis species in their skeletal muscle biopsies, while 6 others were seropositive to *Sarcocystis* (Arness et al., 1999).

In Egypt, the seroprevalance of muscular sarcocystosis in clinically suspected cases ranged from 13% to 15.6% among (RA) patients and from 21.7% to 30% among patients with chronic myositis (El-Nazer & Abdel-Azeem, 2000; Habeeb et al., 1996 and Azab et al., 1990).

Patient history in the myositic form of Sarcocystosis includes painful muscle swellings accompanied by erythema, muscle tenderness, generalized muscle weakness, and fever. Bronchospasm can also occur. Cardiac involvement is asymptomatic (Kiel, 2002).

Aim of Work:

The study of the relation of non-specific rheumatic diseases associated with myositis and RA with tissue *Sarcocystis* infection.

MATERIALS AND METHODS

This study was performed on forty-three patients selected from the Rheumatology & Rehabilitation Out-patient Clinic of Assiut University Hospital. They were considered as two groups:

Group (A):

Consisted of 22 non-specific rheumatic patients (19 females and 3 males) with mean age 20.5 years. They were divided according to the symptoms to two subgroups:

- i) Thirteen cases with arthritis
- ii) Nine cases with arthralgia

Seventeen patients of the two subgroups had myositis. Myositis was diagnosed according to the criteria of the American College of Rheumatology ACR (*Bohan & Peter, 1975*).

Group (B):

Twenty-one RA patients, (20 females and one male).

Ten healthy persons were included as a control group. They were both clinically and serologically negative. Several investigations were done to exclude other rheumatic diseases. Traumatic, degenerative and specific rheumatic disorders were excluded clinically.

Methods:

The serum samples of the selected patients were tested as antistreptolysin O titer, rheumatoid factor, Antinuclear antibodies, serum uric acid, creatine phosphokinase.

Differential blood count for the detection of eosinophilia

Serum samples of group A, B and the control group as well as serum samples from previously proven –ve and +ve *Sarcocystis* cattle (as control) were tested for the presence of *Sarcocystis* antibodies with the Western Blot assay.

Antigens:

Sarcocystis cystozoite Ag. was prepared from *S. fusiformis* as described by *Morsy et al. (1994)*.

Sodium dodecyl sulphate poly acrylamide Gel electrophoresis (SDS PAGE):

Sodium dodecyl sulphate polyacrylamide Gel electrophoresis was done according to the method of *Laemmli (1970)* using a 4% stacking gel and 10% or 12% separating gel. *Sarcocystis* Ag. was diluted 1:2 in a sample buffer and boiled at 100°C for 5 minutes. Fifteen µl of the diluted sample in sample buffer were applied to SDS-PAGE gel at 150 volt. Following separation, proteins were electrophoretically transferred from polyacrylamide gel to immobilon-p transfer membrane (*Millipore Corporation, Bedford, USA*).

Western Blotting:

Membranes were cut into strips and blotted proteins were analyzed by the method of *Towbin et al. (1979)*. All serum samples were tested against *Sarcocystis* Ag. with Western Blot at a dilution of 1:25 in PBS-0.05% Tween, followed by alkaline phosphatase labeled rabbit anti-human IgG (h & L chain) (Sigma). Antigens were revealed by developing the strips using 3,3',5,5' tetra methylebenzidine (TMB; *Kirkegaard and Perry Laboratories, USA*). Molecular weight estimates were made by comparing the motility of the tested samples with that of the standard protein mixture (*Amersham pharmacia biotech., USA*) separated on the same gel. This test has been evaluated for cross-reactivity to related parasites and is non cross reactive with *Toxoplasma* related species (*Abel-Rahman, 2001*).

RESULTS

Group (A) patients presented with musculoskeletal complaints in the form of arthritis (59.1%) or arthralgia (40.9%) with myositis (77.3%). Most cases of myositis showed a predilection to the quadriceps muscle solely (36.4%) or with other muscles (45.5%). Myositis was diagnosed according to the criteria of ACR (*Bohan & Peter, 1975*).

The duration of rheumatic affection ranged from months to several years. The articular affection was mostly in the big joints especially the knees.

Systematic affections presented as chest infection (18.2%) and myocarditis (13.6%). Activity was measured as moderate increase of Erythrocyte sedimentation rate (ESR) (mean ESR₁ 37.8%, ESR₂ 51.2%) and mild decrease in haemoglobin level (mean 11.2/L). The differential blood count showed eosinophilia that ranged from 7 to 20.

Group (B) RA patients (21) was diagnosed according to the criteria of ACR (Arnett *et al.*, 1988). Myositis of this group occurred less frequently (28.6%).

Detection of *Sarcocystis* antibodies with Western Blotting:

Several bands that ranged from 20 kD to 66 kD were detected by the +ve tested patients' serum samples. The most reactive bands were the wide band 23-24 kD and the deeply stained band at 26 kD. The strength of the reaction of the serum with the protein bands is shown on (Fig.1) table (1, 2).

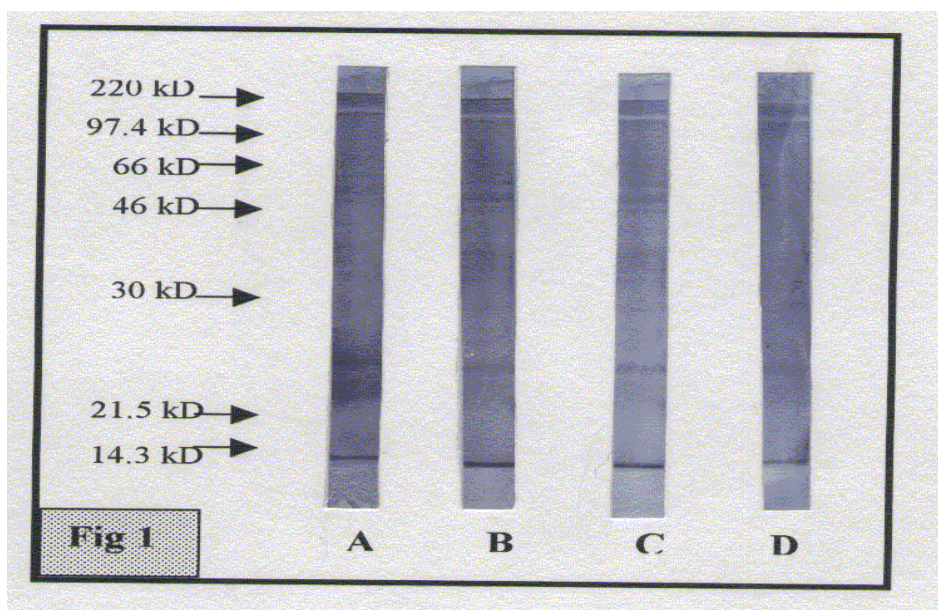


Fig. (1): Four strips of Western Blot test for different degrees of seropositivity.

All the seropositive cases had eosinophilia ranged from 7-20. The 3 patients who complained of myocarditis were positive for *Sarcocystis* Ag.

Table (1): Results of detection of *Sarcocystis* antibodies by Western Bolt in non-specific rheumatic patients.

Serum analysis	No	Percent
-ve	8	36.3%
+ve	10	45.5%
++ve	2	9.1%
+++ve	2	9.1%
Total	22	100%
Control	5	100%

*+ve = weak reaction, ++ve = moderate reaction, +++ve=strong reaction

Table (2): Results of detection of *Sarcocystis* antibodies by Western Blot in RA patients.

Serum analysis	No	Percent
-ve	16	76.2%
+ve	5	23.8%
Total	21	100%
Control	5	100%

Statistical analysis revealed that there was a significant difference between the 2 groups ($p < 0.04$) as shown in (table 3).

Table (3): Statistical analysis of the two groups. Serum analysis cross table between groups A and B (p value < 0.04 sig.).

Serum analysis		Group		Total
		A	B	
(0=-ve) count		8	16	24
	% within group	36.4%	76.2%	55.8%
	% of total	18.6%	37.2%	55.8%
(0=+ve) count		10	5	15
	% within group	45.5%	23.8%	34.9%
	% of total	23.3%	11.6%	34.9%
(0=++ve) count		2	—	2
	% within group	9.1%	—	4.7%
	% of total	4.7%	—	4.7%
(3=+++ve) count		2	—	2
	% within group	9.1%	—	4.7%
	% of total	4.7%	—	4.7%
Total count		22	21	43
	% within group	100%	100%	100%
	% of total	51.2%	48.8%	100%

As regard to the relation between the seropositivity of the non-specific rheumatic arthritis or arthralgia to myositis statistical analysis showed a significant relation as shown in table 4.

Group (A): Showed 59.1% arthritis. Of them 36.4% had myositis and were +ve for sarcocyst Ag. and the last are -ve. p value was significant (0.02). Arthralgia (40.9%) occurred with myositis (27.3%) and was +ve for sarcocyst Ag. and the last are -ve. p value was significant (0.02). Myositis

occured with a ratio of 77.3%, 63.6% of them were +ve for sarcocyst Ag. p value was significant (0.01).

Group (B): Showeed that serum analysis was +ve for sarcocyst Ag. only in 23.8%. Of these, RA cases 9.5% had myositis with +ve sarcocyst Ag. while 14.3% had myositis with -ve sarcocyst Ag. p value was non-significant (0.357) (Table 4).

Table (4): Non-specific rheumatic arthritis or arthralgia in relation to myositis and positivity to sarcocystis antigen.

Cases	No	Ag. Sero. +ve or -ve with myositis	%	p value
Arthritis	13 (59.1%)	8 with myositis +ve sarc 4 without myositis –ve sarc 1 with tendonitis –ve sarc	36.4% 18.2%	0.02
Arthralgia	9 (40.9%)	6 with myositis +ve sarc 3 without myositis –ve sarc	27.3% 13.6%	0.02
Myositis	17 (77.3%)	14 +ve sarc	63.6%	0.01
RA cases with relation to myositis and sarcocystis antigen				
Cases	No	Ag. Sero. +ve or -ve with myositis	%	p value
Rheumatoid arthritis	21	2 with myositis +ve sarc 3 with myositis –ve sarc	9.5%	0.357

DISCUSSION

In the present study Western Blot was used for the detection of *Sarcocystis* antibodies among patients selected from the Rheumatology Outpatient Clinic of Assiut University Hospital. Since there was a remarkable degree of cross reaction among *Sarcocystis species* from widely divergent host origins (Tadros *et al.*, 1981) *Sarcocystis fusiformis* was used as a source of Ag. because they are more available since they are macroscopic, easy to be collected and yield a considerable amount of antigen. This Ag. was used by several researchers. Habeeb *et al.* (1996) and El-Nazer & Abdel-Azim (2000) used *S. fusiformis* Ag. in ELISA and IFAT for the detection of extra-intestinal sarcocystosis in humans.

Western Blot was used for the detection of antibodies against *Sarcocystis* by Arness *et al.* (1999) and El-Nazer & Abdel-Azim (2000) and proved to be accurate and specific.

In the present study the over all seropositivity among the examined serum samples was 46%. When calculated for each group separately, it was 63.7% in the non-specific rheumatic patients and 23.8% in RA patients. These results are higher than those obtained by *El-Nazer & Abdel-Azim (2000)* and *Habeeb et al. (1996)*, who recorded 21.7%, 30% among patients with chronic myositis and 15.6% , 13 % among RA patients respectively.

This high rate of infection in clinically chosen cases is not surprising when compared with random surveys on muscular sarcocystosis. A survey in Denmark by *Greve (1985)* on 112 specimens of human muscle using the trichoscopic technique detected 4 cases of *Sarcocystis* infection (3.6%). Muscle tissue examinations in Malaysia were positive in 21% of 100 consecutive routine autopsy samples (*Wong & Pathmanathan, 1992*) and 19% of Malaysian population sample had serologic evidence of sarcosystosis exposure (*Thomas & Dissanaik, 1978*). This suggested that human sarcocystosis may be much more spread than previously thought and have been overlooked as a zoonosis (*Greve, 1985*).

The low sarcocyst Ag. positivity in RA patients may be due to immunosuppression or use of steroids as said by *El-Nazer and Abdel-Azim (2000)*.

In a study on human sarcosystosis, *El-Nazer et al. (2000)* detected 4 bands at 180, 100, 62, 52, and 3 other bands that were strongly recognized by the infected human sera at the level of 116, 32 and 28 kD. More or less similar results were obtained in the present work, but the most reactive bands were at 116, 100, 52 and a wide band at 32 kD.

Infected patients with *Sarcocystis* develop myositis, characterized by myalgia, localized painful muscular swelling, slight fever, weakness, bronchospasms and eosinophilia (*Pamphlett & O'Donoghue, 1990*). *Arness et al. (1999)* reported symptoms accompanying *Sarcocystis* infection in seven members on a 15-man US military team that operated in rural Malaysia. Symptoms were fever, bronchospasm, Similar symptoms were noticed in the seropositive patients of the present study.

In this study, 17 out of 22 non-specific rheumatic patients complained of fever, myalgia or myositis, arthralgia or arthritis, chest or cardiac problems were positive to *sarcocystis* Ag. The statistical analysis showed that there was a relation between the seropositivity to sarcocystis Ag. and myositis p value =0.01.

Differential blood picture of the seropositive cases expressed eosinophilia that ranged from 7-20. *Arness et al. (1999)* reported peripheral

blood eosinophilia that ranged from 9% to 19% in *Sarcosystis* infected persons and suggested that *Sarcocystis* might be an overlooked cause of unexplained eosinophilia. They identified *Sarcocystis* as the etiologic agent of eosinophilic myositis in their case reports.

Three patients on this study complained from myocarditis and they were positive to *sarcocystis* Ag. one of them reacted strongly with the Ag.

Conclusin:

Sarcocyst infection may be an important cause of non specific rheumatic diseases associated with myositis. Sarcocyst Ag. seropositivity is marked with myositis not arthritis or arthralgia. Non-specific rheumatic patients must be tested for eosinophila and Western Blotting.

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عدوى الساركوسست العضلي في مرضى الروماتيزم غير محدد التشخيص ومرضى الرثيان المفصلي

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يعتبر مرضى الساركوسست العضلي عدوى طفيلية مكتسبة عن طريق أكل لحوم ملوثة بحويصلات الساركوسست.

ولقد أجريت تلك الدراسة على مجموعتين من المرضى:

(أ) 22 مريض روماتيزم غير محدد التشخيص .

(ب) 21 مريض بالروماتويد، و 10 حالات ضابطة.

وباستعمال مولدات مضادات الساركوسست فيوزيفورم اختبرت أمصال المرضى والحالات الضابطة لوجود الأجسام المضادة للساركوسست عن طريق اختبار التحميل المناعي. الإيجابي من المجموعة الأولى 14 مريضاً (63.7%) والإيجابي من المجموعة الثانية 5 مرضى (23.8%). وقد أظهرت هذه الحالات نسبة عالية من الإيوسينوفيليا من 7-20 أما الحالات الضابطة فكانت سلبية.

وإحصائياً الفرق بين المجموعتين كان ذو أهمية إحصائية.

ومن هذه النتائج نستنتج أن عدوى الساركوسست من الأسباب المهمة للالتهاب العضلي المصاحب للروماتيزم الغير محدد التشخيص.

ويجب عمل اختبار التحميل المناعي للمرضى وكذلك نسبة الإيوسينوفيل بالدم.