LYME DISEASE IS NOT PREVALENT IN PATIENTS PRESENTING WITH ANNULAR ERYTHEMA IN SINGAPORE

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ABSTRACT

The aim of this study was to ascertain the prevalence of Lyme disease as a cause of annular erythematous eruptions in patients attending the National Skin Centre. Serum samples of 72 patients presenting with annular erythema were tested for syphilis, and antibodies against Berrelia burgdorferi by haemagglutination, indirect immunofluorescence and enzyme-linked immunosorbent tests. All the serum tested were negative for syphilis serology and antibody against B. burgdorferi. It would appear that the Lyme disease is not a cause of annular erythema in Singapore.

Keywords: *Borrelia burgdorferi infection, spirochaete infection, tick bites*

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INTRODUCTION

Lyme disease is an infection caused by the spirochaete, *Borrelia burgdorferi*. The disease is transmitted by bites from ticks of the *Ixodes, ricinus* complex, infected with *B. burgdorferi*. The animal host of the tick is the white tailed deer. It first came to prominence with the outbreak of a juvenile arthritis-like illness in Lyme, Connecticut, USA in 1977[1]. Today the disease has spread worldwide and has been diagnosed in Europe, some Asian countries and Australia[2]. Cutaneous lesions are the commonest presentation of early Lyme disease. The characteristic cutaneous sign of Lyme disease, erythema (chromicum) migrans presents as localised annular urticarial erythema on sites of tick bites. Tick bite is common in Singapore, particularly amongst the National Servicemen. Some patients with tick bites present with annular urticarial eruptions which mimic erythema migrans (EM). There are numerous other causes of annular erythema which may mimic EM including eczema, collagen disease, insect bite reactions, drug eruptions, urticaria, etc. The tick and animal host for *B. burgdorferi* is not known to be present in Singapore. They are however present in Asian countries including China, Korea, Japan and Taiwan.

We are not aware of any report of Lyme disease in Singapore. We conducted this study to ascertain the sero-prevalence of antibody to *B. burgdorferi* as evidence of Lyme disease, among patients presenting with annular erythema at the National Skin Centre. Lyme disease which may cause severe morbidity is responsive to antibiotics therapy in the early phase of the disease.

Lyme disease can be confirmed by serology tests. The most commonly used serologic tests for detecting antibody to *B. burgdorferi* are: (a) indirect immunofluorescence (IF) test, and (b) enzyme-linked immunosorbent assay (ELISA)[3,4]. False positive results may occur in healthy subjects and in patients with other diseases including syphilis, autoimmune diseases and neurologic disorders[5]. In the present study, an ELISA test for antibody against *B. burgdorferi* was compared with the IF test and the passive haemagglutination (PHA) test.

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MATERIALS AND METHODS

Specimen collection

Patients presenting with annular erythema (with or without history of insect bites) at the National Skin Centre between June 1993 and May 1994 were included in the study. Annular erythema is defined as ring or circinate lesions with erythematous margin persisting for more than 24 hours. The margin may be flat or macular or raised or papular. The lesions may be located on any part of the body. 5 mL whole blood were collected from these patients.

The serum samples were collected and stored at -20°C prior to test.

Serological test methods

(1) Enzyme-linked Immunosorbent (ELISA) Test (3M IgG/IgM Fastline Test. 3M Diagnostic System, Inc, Santa Clara, California). The ELISA test for antibody against *B. burgdorferi* was conducted in the standard method based on the supplier's instruction. Serum reference controls were included in every run. The results interpretation were given as follows:

Negative ≤ 8.5% of serum reference
Borderline = 8.5% - 11.5% of serum reference
Positive = 11.5% of serum reference
High Positive ≥ 25% of serum reference

(2) Passive Haemagglutination (PHA) Test. (Lymix, Diagast Laboratories, Lille Cedex, France). A serodiagnosis by passive haemagglutination test was used as a second test. The test was performed in the standard according to the suppliers' instructions. The erythrocytes used in the assay are sensitised after treatment with an antigen made of cellular components from a strain of *B. burgdorferi*. The presence of specific antibody against *B. burgdorferi* induces the passive agglutination of the sensitised red cells.

(3) Indirect Immunofluorescence (IF) Test. (Lymag. Diagast Laboratories, Lille Cedex, France). An indirect immunofluorescence test was used as our third test. The patients' sera were reacted with *B. burgdorferi* fixed on a slide according to the manufacturer's specification without

modification. Specific reactive antibodies were observed when viewed under the fluorescence microscopy.

Because of the unavailability of confirmatory test eg Western blot tests and culture tests, and for consistency for diagnosis (of positive serology test), we arbitrarily considered serology test for *B. burgdorferi* to be positive if at least two of the serology tests are positive.

All the serum were also tested for syphilis serology with the treponema pallidum haemagglutination test (TPHA test kit, Fujirebio Inc, Tokyo) and RPR card test (Syphilis RPR-test, Human Gesvllschaft, Taunusstein).

RESULTS

Sera from 72 patients were included into the study. None of the patients had history or clinical signs of tick bite reaction. Of the 72 sera tested by ELISA method, 5 were found within the range of 12% to 14% of serum range positivity. The cut-off point for positivity was >11.5%. When these samples were tested by passive haemagglutination and indirect immunofluorescence tests, all were found to be negative. The remaining sera all tested negative by ELISA, passive haemagglutination and indirect immunofluorescence tests. The syphilis serology were negative for all the 72 samples.

DISCUSSION

The spirochaete, *Borellia burgdorferi* is now recognised as the organism responsible for the multisystem disorder, Lyme disease[6]. Lyme disease runs a variable course. Only about 1%-3.5% of bites from infected tick bites resulted in Lyme disease[7]. The initial stage of the disease presents as localised migratory annular erythema of about 5 cm in diameter at the site of tick bite. The lesion may be asymptomatic or associated with burning sensation or pain. Atypical lesion may be confluent, vesicular, purpuric or eczematous. Minor constitutional symptoms, fever and regional lymphadenopathy may occur. Lyme disease is responsive to systemic antibiotics (including amoxicillin 500 mg tds or doxycycline 100 mg bd for 10-30 days) during the early phase of the disease. If left untreated, Lyme disease enters into a chronic stage.

The second stage of Lyme disease (disseminated disease) presents with neurologic, rheumatologic and cardiac symptoms as these organs become affected. Secondary annular cutaneous lesions may appear. The third stage of Lyme disease (persistent disease) presents as acrodermatitis chronica atrophicans which manifest as localised scleroderma-like skin lesions with prolonged neurologic and rheumatologic symptoms[8,9].

The diagnosis of the Lyme disease can be confirmed only with the availability of antibody tests against *B. burgdorferi* and culture tests. To date, several serology tests are available to detect antibody against the organism. Antibodies to *B. burgdorferi* are detected either by an enzyme-linked immunosorbent assay or an indirect immunofluorescent antibody staining. Passive haemagglutination technique has recently been developed for the detection of antibody to *B. burgdorferi*[4]. The sensitivities and specificity of these tests are still controversial[4]. IgM ELISA antibodies to *B. burgdorferi* titres peak 3-6 weeks after infection. IgG antibodies peak at 3-6 months after infection.

In our study, we have used three serology tests for *B. burgdorferi* antibody. This was necessary to minimise false positive or false negative test. The Western blot test was not available for us to conduct confirmatory test. Our findings showed that the ELISA test appeared to be less specific than the indirect immunofluorescence test and the passive haemagglutination test. The five false positive serology from the ELISA tests showed borderline positivity only. This could represent a calibration problem due to low cut-off reading of our positive reference serum. There is therefore a need to conduct confirmatory tests using either IF or PHA test when confronted with a positive ELISA test for Lyme disease. Alternatively, the Western blot test which is less readily available should be used to confirm positive ELISA[10].

All the 72 sera of patients presenting with annular erythema tested negative for *B. burgdorferi* antibody. It would appear that Lyme disease is an uncommon cause of annular erythema in Singapore at present. However, there is a need to be vigilant on the occurrence of Lyme disease in South East Asian countries as the tick vector for *B. burgdorferi* is prevalent in this part of the world.

Failure to detect *B. burgdorferi* antibody in our patients could be due to false negative serology tests. The availability of more sensitive tests might help to improve detection rate. The polymerase chain reaction (PCR) techniques may help to improve sensitivity of diagnostic tests for Lyme disease[11]. Only a small number of spirochactes are usually present in skin lesions or body fluids in infected patients. PCR techniques may enable the clinician to confirm the diagnosis of Lyme disease more accurately.

Laboratory diagnosis has been problematic because of lack of sufficient sensitivity and specificity of many tests used as well as the lack of inter-laboratory standardisation in the performance of these tests. Although helpful, the laboratory tests can only serve to support the clinical findings in making the diagnosis of active Lyme disease, the exception being a positive culture for *B. burgdorferi*[4]. The diagnosis of Lyme disease has to be based on the classical clinical features. Atypical presentation should be watched for.

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