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LYME BORRELIOSIS: CLINICAL MANIFESTATIONS AND ADVANCES IN DIAGNOSIS AND CONTROL

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ABSTRACT

Lyme disease or borreliosis is presumed one of the most significant vector-borne diseases globally. The disease is re-emerging in numerous parts of world. It has expanded dramatically in newer areas in recent decades. Lyme disease is caused by *Borrelia burgdorferi* yet additionally by other borrelial species, *B. afzelii* and *B. garini* which cause diverse clinical syndromes. Spatial distribution and clinical presentations differ depending on the causative species. Clinical manifestations of Lyme disease can be delineated in three stages. The first stage is presented in the form of erythema migrans at the site of tick bite. Early dispersed stage can lead to multiple lesions of erythema migrans, neuroborreliosis, lymphocytoma, arthritis or carditis. The manifestation at later stage shows acrodermatitis chronica atrophicans, arthritis and neurological involvement. Diagnosis is challenging owing the several clinical presentations and could require multiple tests. The antibiotics that are currently under use, help in the clearance of bacteria from the affected host and stop further spread of the disease. Although several antibiotics are being used for Lyme disease, doxycycline is the widely used antimicrobial in early stage of the disease. Several attempts have been made to develop a vaccine against Lyme disease, however, none of them have been successfully marketed. The present review discusses clinical manifestations, and advances in diagnosis and control of Lyme disease.

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1 Introduction

The Lyme disease (LD) was initially found at Old Lyme; Connecticut, in the year 1975. The affected group of patients was suffering with some uncommon symptoms of arthritis. By the ending of 1977 further 51 cases of “Lyme arthritis” were observed (Saccomano & Hrelac, 2018). Lyme borreliosis (LB) or Lyme disease, is a developing and re-emerging tick borne disease caused primarily by *Borrelia burgdorferi* sensu stricto in Europe and North America by *B. afzelii* or *B. garinii* (Smith & Thackinen, 2006; Bacon et al., 2008; Hubalek, 2009; Nelson et al., 2015; Schwartz et al., 2017; Sykes & McIllo, 2017). The disease was mostly observed in similar climatic conditions of Scandinavia and Central Europe, the north eastern and Middle Western areas of United States as well as in some parts of Asia. The disease is reported to be Zoonotic and may be transmissible to humans through *xodes* ticks (Russell et al., 2018). Generally, *B. burgdorferi* is one of the most potent species causing joint infection, *B. afzelii* causes a typical skin infection, acrodermatitis chronic atrophicans (ACA) (Maraspin et al., 2019), and *B. garinii* most often causes neurological manifestations (Steere et al., 2016). Lyme disease is usually curable with treatment. If undiagnosed or incorrectly diagnosed may leads to debilitating conditions in patients. This may further enhance the cost of treatment (Mac et al., 2019).

Lyme disease has now attained public health concern worldwide. In Lyme (Connecticut) the affected children were found to be affected with juvenile rheumatoid arthritis (Van Hout, 2018). In humans, the disease is transmitted when ticks ingest the human blood which leads into the transformation of its further stage of development. While attaching to the human host; the ticks insert its hypostome into host skin. The *Ixodes* ticks secrete anticoagulants, anti-inflammatory as well as immunosuppressive chemicals which facilitates pathogen to enter into the host body (Carriveau et al., 2019).

The clinical symptoms of Lyme disease occur in stages (Steere, 1989). The majority of patients witness the development of the disease with erythema migrans which is a rash developing at the tick bite site. During early stage infection could be limited to the skin or spread through hematogenous route with inclusion of the skin, nervous system, heart or joints. Some untreated patients develop advanced stage disease within weeks or months, chiefly Lyme arthritis (Steere, 1989). Practically, most of the patients during early phase of disease could be treated with a short duration course of appropriate antibiotic regimen given orally, however the early stage of disease is not overt. In later stage, many persons could be treated successfully with antibiotic therapy yet a few patients do not respond to antibiotics, therefore require other management strategies (Steere & Angelis, 2006; Arvikar & Steere, 2015).

Diagnosis of LB is predicated absolutely on serological tests and PCR, yet establishing the diagnosis are often complicated especially at the early stages before the development of immunological response and for infection spreading to central nervous system, heart or joints. Moreover, downside of serological testing is that antibodies persist for

quite a long time following infection. Moreover, scientists have sought to develop elective tests for serology, PCR and culture to accurately and timely diagnose and monitor the early and late stages of disease, even in people with explicit symptoms. The present review highlights the clinical manifestations, and advances in diagnosis and control of Lyme disease.

2 Clinical Manifestations

The clinical appearance of Lyme disease can be categorised in three stages to simplify its diagnostic procedure. Early localized stage is portrayed by erythema migrans at localized site of bite of tick. The subsequent stage strikes from weeks to months post-exposure and is manifested by multiple erythema migrans lesions, borreliolymphocytoma, neuroborreliosis, carditis or infrequently Lyme arthritis. The third stage is characterized by acrodermatitis chronic atrophicans, neurological symptoms and arthritis (Borchers et al., 2015). A second thought of the chronic form of LD is also prevalent among the research workers. They designate it as Post Lyme Syndrome which is also an inflammatory reaction phenomenon may be because of the persistence of the bacteria; probably due to Biofilm formation despite proper antibiotic therapy (Lacout et al., 2018). The existence of these signs and symptoms are species specific and differ between geographical regions.

2.1 Cutaneous/Dermatologic manifestations

There are mainly three characteristic skin manifestations which include erythema migrans, lymphocytoma and finally acrodermatitis chronica atrophicans (ACA). Erythema migrans appears in acute phase, whereas, lymphocytoma is a subacute manifestation and acrodermatitis is a chronic culminating manifestation in later stages of disease.

2.2 Erythema migrans

The most widely recognized form of Lyme borreliosis is erythema migrans, which extends from few days to few weeks with red or blue-red macular skin lesions (Bergland et al., 1995; Strel et al., 1996). Conversely, tick bite rashes develop from hours to days and usually fade in certain days. In young children, the most influenced areas are the neck and head; pelvic region or extremities in adults (Borchers et al., 2015). Systemic symptomatic presentation such as pain, fever and lymphadenopathy may occurred along with erythema migrans. Just about portion of the individuals who experience the ill effects of erythema migrans recollect the tick bite. *B. burgdorferi* and *B. garinii* often result in to homogeneous erythema migrans (Bennet et al., 2006a; Bennet et al., 2006b; Vasudevan & Chatterjee, 2013). Lesions persevere from weeks to months if remain untreated (Steere et al., 1987; Mirafior et al., 2016).

2.3 Borreliolymphocytoma

Borreliolymphocytoma is an unusual cutaneous symptoms characterized by painless blue-red nodules and is principally observed in children (Vasudevan & Chatterjee, 2013; Arnez & Ruzic-Subljic,

2015). Generally, borrelial lymphocytoma is apparent on the ear lobe, nipple or scrotum. There is usually a concomitant erythema migrans or is followed after a stage of erythema migrans (Berglund et al., 1995; Maraspin et al., 2016). Predominantly, *B. afzelii* causes borrelial lymphocytomas but *B. burgdorferi* and *B. bissetti* have also been reported (Maraspin et al., 2016). With antibiotics, borrelial lymphocytoma usually clears up within several weeks with treatment. Histopathology shows lymphocytic infiltration with plasma cells and germinal centres into the skin (Borchers et al., 2015). The differential diagnosis has a broad spectrum and comprises lymphomas, insect bites and sarcoidosis. Biopsy is often required in non-localized areas. The diagnosis can be affirmed by the historical backdrop of erythema migrans, serological tests, and molecular methods such as PCR, organism culture or isolation on biopsy examination.

2.4 Acrodermatitis chronica atrophicans

Acrodermatitis chronic atrophicans is a cutaneous manifestation associated with late stage of Lyme disease that is generally reported in European countries and is chiefly caused by *B. afzelii* (Asbrink et al., 1986). ACA shows up as a chronic, gradually progressive reddish or bluish cutaneous lesion that may later on become atrophic (Asbrink & Hovmark, 1988). ACA is the late manifestation of Lyme borreliosis and may last for months to years if untreated (Ogrinc et al., 2017). ACA has comparatively more prevalence in aged women and prefers the extensor surface of the distal extremities (Steer et al., 2016). Lesions start as edema with bluish-red plaques that gradually develop into atrophic and hyperpigmented plaques (Godar et al., 2015). Over half of patients report peripheral neuropathy equivalent to allodynia (Mullegger & Glatz, 2008). ACA has been demonstrated to be unexpectedly resolved unlike other manifestations of Lyme borreliosis (Asbrink & Hovmark, 1988). Indeed, lesions more than 10 years of age may likewise be exposed to active infection by culture or PCR positivity and antimicrobial therapy responsive (Agterof & ter Borg, 2008; Lenormand et al., 2016). The presence of typical symptoms similar to granuloma annular, lichen sclerosis or pseudolymphoma can interfere with the diagnosis. In addition, recent reports include newer clinical forms of the ACA, which could be spinous papules with erythema, small violaceous patches and foot tumour (Lenormand et al., 2016; Bauvin et al., 2017).

2.5 Lyme neuroborreliosis (LNB) / Nervous system manifestations

Borrelia spp. mainly affect skin, joints, nervous system or in some instances heart. Neurological form known as Lyme neuroborreliosis (LNB) have been accounted in one tenth of Lyme borreliosis cases (Berglund et al., 1995; Halperin, 2015). Early Lyme neuroborreliosis usually endures from days to weeks following a tick bite with lymphocytic meningitis, radiculoneuritis or cranial neuritis (Halperin, 2013; Koedel et al., 2015; Ogrinc et al., 2016). LNB is more common in children with facial nerve paralysis (Sodermark et al., 2017), whereas adults show radiculoneuritis and lymphocytic CSF pleocytosis (Hansen

& Lebech, 1992; Koedel et al., 2015). CSF pleocytosis, intrathecal specific antibodies and neurological features help in the affirming the diagnosis of *B. burgdorferi* infection (Zajkowska et al., 2015).

2.6 Lyme arthritis

Lyme arthritis usually occurs as oligo or monoarthritis more frequently in the knee joint about three to six months post infection (Steere et al., 1987; Arvikar & Steere, 2015). Lyme arthritis is less agonizing and is not accompanied by pyrexia unlike other causes of septic arthritis. Most of the patients do not report previous tick bites or erythema migrans. Manifestations can include intermittent or prolonged joint swelling and pain for months to years in untreated patients. Arthritis can be cured and borreliosis can be eradicated in most patients with proper antimicrobial treatment, in spite of the fact that at times proliferative synovitis may last for months to years together warranting anti inflammatory medicines (Arvikar & Steere, 2015; Grillon et al., 2018). Anti-Borrelia antibodies detection, occurrence of erythema migrans earlier or detection by PCR in synovial fluid warrants appropriate therapy. Most of the arthritic patients could be cured by appropriate antibiotic therapy and nonsteroidal anti-inflammatory drugs (Mullegger & Glatz, 2008). These complex cases may benefit from treatment by methotrexate and hydroxychloroquine like other chronic inflammatory arthritis (Arvikar & Steere, 2015).

2.7 Lyme carditis / cardiovascular manifestations

Acute cardiovascular inclusion may happen, which is portrayed by varying degrees of atrioventricular conduction defects in early disseminated infection (Lelovas et al., 2008; Steere et al., 2016). Some of the infrequent cardiac manifestations could include cardiomyopathy and acute myopericarditis. The Lyme carditis may also be manifested with several other conditions like acute myocarditis, pericarditis, myopericarditis, endocarditis, and pancarditis. Such conditions may leads to the reduced left ventricular cardiac functions. The Acute Coronary Syndrome may mimic with the Lyme myopericarditis (Yeung & Baranchuk, 2019). The most frequent manifestations in patients having Lyme borreliosis with involvement of the heart are Atrioventricular Conduction disorder or other rhythm disorders, myocarditis, pericarditis, Postural Orthostatic Tachycardia Syndrome (POTS) (Trevisan et al., 2020). Lyme carditis is often self-limiting yet might be fatal if there is no treatment (Robinson et al., 2015; Scheffold et al., 2015).

3 Diagnosis and Advancements in Diagnosis

Diagnosis may be possible by isolation and identification of *B. burgdorferi* from the lesions of erythema migrans and the blood of affected persons especially during early stages (Steere et al., 2016). Center for Disease Control has recommended a two step assay. The first step starts with, the enzyme immunoassay or immunofluorescence assay. The positive assay of this test is subjected to Western blot analysis (CDC, 1995). Erythema migrans

can be diagnosed dependent on clinical outcomes including exhibited or suspected tick bites or local site exposure. The IgM isotype is positive in one-fifth to half of cases in the initial two to three weeks and IgG is positive in one to two months post infection (Steere et al., 2016). Borrelial lymphocytoma diagnosis is upheld with positive serological results. Histopathology could be helpful in the diagnosis and is regularly done to differentiate from other conditions. Moreover, biopsy sample analysis by PCR or culture confirms the diagnosis, in spite of the fact that the sensitivity of these tests is highly variable. ACA is characterized by high-titer serum IgG antibodies. Although, high-titer tests are usually adequate; histopathological analysis, PCR or tissue culture may help in diagnosis (Stanek et al., 2011). Lyme arthritis with high-titer IgG provides *Borrelia* antibodies. The two-tier analysis approach is extremely useful and sensitive (Shapiro, 2014). To confirm Lyme neuroborreliosis (LNB) diagnosis specific clinical manifestations along with intrathecal specific antibody production are authentic parameters. Cerebrospinal fluid (CSF) pleocytosis is quite often present. Blood and CSF ought to be analyzed for antibody detection and profiling with the demonstration of central nervous system (CNS) antibody production. In early stages of LNB, intrathecal IgG is positive 6 to 8 weeks before the onset of symptoms (Stanek et al., 2011). Culture and PCR have low yields in LNB but could be helpful for diagnosing early antibody negative cases. The new biomarker chemokine CLCX13 has additionally shown a promise, however, its application has not been recommended so far.

There are limited reports of cultural isolation from synovial fluid however, *B. burgdorferi* DNA could be detected in synovial fluid by PCR and also with lesser reliability from the CSF (Steere et al., 2016). In Europe, for the diagnosis of Lyme neuroborreliosis, testing for intrathecal antibody production is comprehensive where multiple *Borrelia* spp. and high seroprevalence limit the use of serological analysis (Stanek et al., 2012). Two-tier serological analysis is the diagnostic test of choice in most cases of LNB (Aguero-Rosenfeld et al., 2005; Wormser et al., 2005; Wormser et al., 2006). Adjunctive testing for intrathecal antibody production is quite specific in diagnosing the disease especially in endemic regions. When testing for intrathecal antibodies, it is imperative to observe that in some patients with Lyme disease, antibodies in the serum are passively transferred to the CSF (Steere et al., 1990). To check this transfer, CSF and serum should be collected simultaneously and the concentration of total protein or IgG should be matched.

The novel ELISA technique; capable to detect early IgG in suffering suspected patients of Erythema migrans, is under development. The availability of ELISA test using C6 peptide as an antigen of VlsE lipoproteins of *B. burgdorferi* was found to be useful because of its higher sensitivity for *B. afzelii* and *B. garinii* (Bush & Vazquez-Pertejo, 2018).

PCR provides unique evidence of *B. burgdorferi* nucleic acid in diverse samples including skin biopsy, synovial fluid, blood and CSF (Babady et al., 2008). Though, its clinical utility is restricted due to its low sensitivity and its vulnerability for contamination (Molloy et al., 2015). Synovial fluid PCR is highly sensitive to Lyme arthritis and can be used in combination with other synovial fluid analyses to make a differential diagnosis of Lyme arthritis from other arthritis (Babady et al., 2008). Comparatively, the PCR of CSF is significantly less sensitive limiting its clinical applicability. The PCR trials of CSF gave positive outcomes with early neuroborreliosis but less sensitive to late neuroborreliosis (Nocton et al., 1996). PCR for Lyme Diagnosis is not universally standardized or optimized. PCR of blood demonstrated the utility in identifying the novel genus *B. miyamotoi* and *B. mayonii* (Molloy et al., 2015). Detection by PCR is accomplished by amplification of selected genes: 16S rRNA gene, 23S rRNA gene, the 5S rRNA-23S rRNA gene spacer, *p66*, *fla*, *recA* and *ospA* (Aguero-Rosenfeld et al., 2005). Genotyping and quantification of *Borrelia* can be made from skin biopsies using real-time PCR (Liveris et al., 2012; O'Rourke et al., 2013). The strengths of PCR assays in skin samples are due to its high sensitivity, rapidity and direct detection from the original biopsy sample. However, the identification of *Borrelia* DNA does not generally indicate an active or concurrent infection and DNA can be detected at least in mouse specimens after antibiotic treatment (Grillon et al., 2017). Since *B. burgdorferi* is a slow-growing organism, therefore, cultural methods are cumbersome and less sensitive and therefore, are not generally recommended (Aguero-Rosenfeld et al., 2005).

In future, direct techniques for culture, detection of borreliol antigens, specific nucleic acid detection and genetic sequencing could gain importance (Ruzic-Sabljić & Cerar, 2017; Branda et al., 2018; Schutzer et al., 2019). At present, validated diagnostic test for confirmatory diagnosis of *B. burgdorferi* is antibody response to confirm the diagnosis of Lyme disease (Marques, 2015; Theel, 2016). A two-step assay based on the initial ELISA was developed which was followed by Western blot testing for IgM as well as IgG antibodies to increase sensitivity and specificity during various phases and in different forms of Lyme disease (Alasel & Keusgen, 2018). In recent times, the Food and Drug Administration (FDA) approved EIA tests which target the specific immune response against the cell surface variable major-protein-like sequenced expressed (VlsE) along with its sixth invariable region, C6 peptide. These simple tests replace western blot testing (Schoen, 2013). However, the persistent problem is serological over-testing, misinterpretation of tests and over-diagnosis (Webber et al., 2019; Markowicz et al., 2015; Lantos et al., 2015).

Few more rapid diagnostic testing (RDT) are available, of those the important two are: immunochromatography based tests and microfluidic based tests. In Europe, immunochromatography based methods are economically accessible in clinical laboratories and for self use. These RDTs are simple and require no exhaustive specialized training. However, the RDT performance is yet to

evaluate fully, moreover, regulations for these tests are also not available till date (Smith et al., 2014). Another test, the microfluid-based rapid point-of-care test, which could provide results within 15 minutes, is yet to be made available in market (Nayak et al., 2016). This test was 84% sensitive and 92% specific in comparison to laboratory-based C6-peptide ELISA, but less sensitive than recombinant ELISA (Nayak et al., 2016). Alternative diagnostic tools are needed to confirm the active cases. Recently, xenodiagnosis with live ticks has been tested to know the effectiveness of antibiotic therapy (Marques et al., 2014). However, live larval ticks may induce strong itching reactions in some patients, so may be used with due caution. Alternative diagnostic tools are required to identify live forms of *Borrelia*. Mass spectrometry could directly detect pathogenic proteins in the skin. The use of proteomics in the skin has been successfully done, especially in punch biopsy in psoriasis patients (Lundberg et al., 2015) or in patients with keratome biopsy to reduce cellular complication (Williamson et al., 2013).

4 Treatment, Prevention and Control

The most of the cases of neuroborreliosis are curable with time; without treatment. The antibiotics under use, helps further in the clearance of bacteria from the host affected and stop further spread of the disease. This reduces the losses of axonal degeneration. Penicillin G, Doxycycline, Ceftriaxone, Cefotaxime etc are some antibiotics which were found to be effective in early neuroborreliosis. The uses of steroids are also not been reported in neuroborreliosis (Russell et al., 2018). Doxycycline is the widely used antimicrobial in early stage of Lyme Disease. Minocycline is an alternative second choice of drug (Carriveau et al., 2019). An open-label randomized controlled trial was conducted to evaluate the efficacy of single dose doxycycline therapy following tick bite to prevent the development of Lyme borreliosis (Harms et al., 2020). The findings indicate that administering a single dose of doxycycline (200 mg) within 72 hours can prevent the development of Lyme borreliosis following *Ixodes ricinus* tick bite. Although, Lyme borreliosis generally responds very well to antibiotic therapy, patients may develop persistent symptoms, most commonly referred to as chronic Lyme (Kullberg et al., 2020). These symptoms include pain, fatigue, and neurocognitive symptoms that can persist and at the same time highly disabling.

The best prevention for the Lyme disease is to avoid to move in tick infested areas; the areas of tall grasses, shady and moist. Such lands may be of higher risk for infections. If the movements in such areas are unavoidable then one should wear the cloths which fully cover their body, light colored cloths which are less attractive for ticks. People also use diethyltoluamide, commonly named as DEET which works as insects repellent.

At present there is no human Lyme disease vaccine available. Although several attempts have been made to develop a vaccine, none have been

successfully marketed. Proteomic approach has been applied to identify vaccine candidates for Lyme disease from skin biopsies in a mouse model. A total of 30 bacterial proteins were identified after syringe inoculation or tick inoculation of bacteria. Therefore, further investigations should be conducted to define the role of each protein in the elaboration of protective immunity (TalaGrand-Reboul et al., 2020). Recently, structure-based surface shaping approach was used for the development of vaccine against Lyme borreliosis. The proposed multivalent outer surface protein A (OspA) vaccine is expected to target wide range of pathogenic *Borrelia* species with the help of a single recombinant antigen thereby offering an efficient preventive strategy against Lyme borreliosis (Nayak et al., 2020).

Conclusions and Way Forward

Lyme disease has gained renewed importance during last two decades. The disease is always a suspect in endemic areas and can be successfully diagnosed, monitored and treated. However, diagnosis is a problem in areas where the disease has very low prevalence especially due to lack of knowledge about this disease and its manifestations. Skin afflictions are early symptoms of Lyme disease which could eventually lead to arthritis, neurological and cardiac forms in due course. Additionally, PCR, serology and some more diagnostic assays are developed, but their validation or assessment is yet incomplete. More focused research is required to find out their sensitivity, specificity and reproducibility. Identification of new biomarkers to rapidly confirm the disease could be a major breakthrough in its diagnosis and subsequent control of active *Borrelia* infection and could prove to be a major advancement in LB diagnosis and monitoring.

Author contributions

All the authors substantially contributed to the conception, compilation of data, checking and approving the final version of the manuscript, and agree to be accountable for its contents.

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