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Review Article

Microbiology Section

Leptospiral Sphingomyelinase 2 (Sph2): A Multifunctional Virulence Factor in Pathogenesis and Diagnostics

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ABSTRACT

Leptospiral Sphingomyelinase 2 (Sph2) is identified as one of the important virulence determinants and it is a potential biomarker in detecting leptospirosis at its initial stages. Empirical studies have proved that Sph2 is actively released in infection and its activity may be detected in serum and urine of patients. The presence of Sph2 in such biological fluids is an important diagnostic parameter that can be used to differentiate between leptospirosis and other febrile diseases that can have similar clinical features. Furthermore, clinical studies have shown that the levels of Sph2 in the urine of patients with a concurrent infection of dengue and leptospirosis double, which suggests its possible use in the endemic areas with both of these pathogens. The Sph2-based assays have demonstrated sensitivity ranging from 80.6% to 91% and specificities ranging from 67% to 100%, high and as compared to the traditional antibody-based assays, especially in the early stages of infection when humoral responses are yet to be developed. However, there are a few issues, such as strain-dependent fluctuations in the expression of Sph2 and the need to standardise detection protocols. Even with these limitations, integrating Sph2 detection into rapid and easy-to-use diagnostic tools- like lateral flow or biosensor-based assays- could significantly improve the early and accurate diagnosis of leptospirosis, particularly in resource-limited healthcare settings.

Keywords: Biological markers, Haemolysis, Host-pathogen interactions, Urine, Zoonotic diseases

INTRODUCTION

Leptospirosis is a globally distributed zoonotic disease caused by pathogenic *Leptospira* species and is spread from reservoir hosts, usually rodents, to humans through contaminated soil or water. Leptospirosis has a major negative influence on public health and is prevalent in tropical and subtropical areas of the world [1]. Initially, *Leptospira* was only classified into *L. interrogans* and *L. biflexa*, which clearly divide the pathogenic and non pathogenic species. Later on, these two classifications were further divided into specific serovars on the presences of homologous antigen (nearly 60 serovars under *L. biflexa* and at least 225 serovars under *L. interrogans)* [2]. Leptospirosis is a common disease throughout the world, with an estimated one million infections and 60,000 deaths per year [3]. Because of their helical structure and characteristic hook ends, these organisms may be easily distinguished from other spirochaetes. With a thickness of roughly 0.1 to 0.15 µm and a length of 6 to 20 µm, they are long and thin [4]. Leptospires are aerobic and slow-growing organisms that are highly susceptible towards drought and hypertonic conditions [5]. *Leptospira* are Gram-negative spirochaetes with a protein-rich outer membrane and periplasmic flagella that enable motility and contribute to pathogenicity [6]. Pathogenic spirochaetes from the genus *Leptospira* are the source of the illness, and they can infect nearly all animals, humans, reptiles, and amphibians [7]. Leptospirosis presents with a broad spectrum of non specific clinical features, ranging from mild febrile illness to multiorgan failure. Because of this overlap, leptospirosis is often termed a “mysterious mimic” and is frequently misdiagnosed as other febrile illnesses such as dengue, malaria, scrub typhus, rickettsial infections, and melioidosis [8]. Additionally, leptospirosis has a major negative impact on the agriculture sector by resulting in cattle deaths, infertility, and abortions [9].

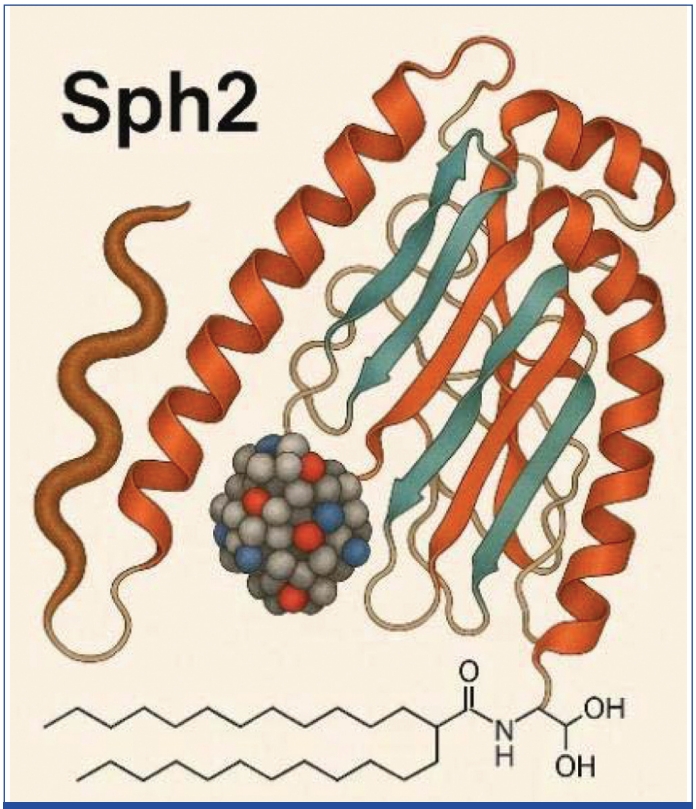
Leptospira differ from other spirochaetes such as *Treponema* and *Borrelia* by an atypical Lipopolysaccharide (LPS)-containing outer membrane that enables the surface expression of virulence factors, including Sphingomyelinase-2 (Sph2), involved in host cell damage, immune evasion, and diagnostic applications [10]. Leptospires are primarily extracellular pathogens that express multiple virulence factors during infection [2]. Despite extensive research, the pathophysiology of leptospirosis remains incompletely understood. The disease is thought to arise from host immune responses to high leptospiral burdens in blood and multiple organs, including the liver, lungs, kidneys, and cerebrospinal fluid [11]. Despite the current diagnostic techniques, non specific clinical symptoms hamper early Leptospiral diagnosis [6]. The Microscopic Agglutination Test (MAT) is the gold standard for diagnosing leptospirosis, but it has several limitations, including the need for a second serum sample, the need to keep live serovars, and the use of complex equipment like a dark-field microscope [12]. Additionally, during the first week of infection, other serological assays, like as Enzyme-Linked Immunosorbent Assay (ELISA) and immunochromatography-based testing, are less sensitive. During the immunological phase, the sensitivity of direct detection by culture, dark-field microscopy, traditional Polymerase Chain Reaction (PCR), real-time PCR, and recently developed assays like Carbo-Lip and E-Lip 32 is reduced [13,14]. These investigations clearly demonstrate that the diagnostic tests that are already on the market have limitations of their own and that their precision in identifying leptospirosis is still lacking. Biomarkers detected in blood and urine samples play a vital role in diagnosis of infection and treatment follow-up [15]. Currently available diagnostics for leptospirosis are either based on biomarkers that indicate the presence of *Leptospira* or antibodies against *Leptospira* [16]. Leptospires generate a number of virulence factors when infected [2]. Sphingomyelinases are of great interest because of their potential to mediate key aspects of Leptospiral pathogenesis. Through their action on host cell membranes, leptospiral sphingomyelinases are potentially involved in aspects of pathogenesis, including tissue invasion, endothelial damage, immune evasion and nutrient acquisition [17]. Leptospiral sphingomyelinase haemolysin, expressed only by pathogenic *Leptospira* serovars, facilitates host cell lysis and nutrient acquisition, aiding bacterial survival and causing damage to the liver, kidneys, and lungs [18].

In this review, it is defined that leptospiral Sph2 is a versatile virulence determinant and that is at the centre of host-pathogen interaction, cellular injury, and immune modulation. The article highlights the future potential of Sph2 in the diagnostics and treatment of leptospirosis, as it has a high antigenicity in-vivo and it secretes a large quantity of Sph2 upon infection. It also compares the structural and functional characteristics that make Sph2 a good biomarker and a potential vaccine target. By synthesising the evidence on Sph2 pathogenesis, diagnostics and immunology, this review provides a complete evaluation of Sph2 and translational applicability in developing strategies to deal with leptospirosis at early stages, through therapeutic intervention and preventive strategies.

Molecular and Structural Aspects of Sph2

Pathogenic Leptospiral organisms express a defined set of virulence determinants that are involved in the range of pathologies that these bacteria cause in the human host [19]. Sphingomyelin (SM) is an important constituent of eukaryotic cellular membranes, and the ability of bacteria to break down this phospholipid can, therefore, be implicated in the pathophysiology of infection. Both eukaryotes have a class of haemolysins called sphingomyelinases, which often function as toxins in the latter and are connected to phospholipid metabolism in the former [20]. Sphingomyelinases are one of these pathogenic agents that are linked to haemorrhagic complications in leptospirosis because they catalyse the hydrolysis of sphingomyelin in host cell membranes. Additionally, these compounds may play a role in nutrition acquisition and immunological evasion [17]. Pathogenic leptospires produce five sphingomyelinases (Sph1, Sph2, Sph3, Sph4, and Sphingomyelinase Haemolysin (SphH)), which are not present in non pathogenic *L. biflexa* [18]. The structural resemblance of Sph2, a Mg++ dependent haemolysin with shown sphingomyelinase activity, to Sphingomyelinase C-like protein (SMcL), the sphingomyelinase from *L. ivanovii*, stands out among these five proteins and the haemolytic process as shown in [Table/Fig-1]. The full-length amino acid sequences are compared demonstrating that the Leptospiral sphingomyelinases had a 186-amino acid C-terminal extension [21].

The Leptospiral sphingomyelinases' 3D structure, as predicted by Insight II Modeler, was made up of a core sandwich architecture with loops and helices that matched those of SmcL and Bacillus cereus Sphingomyelinase (BC SMase), which were utilised as templates. A comparison of the full-length amino acid sequences revealed that the Leptospiral sphingomyelinases (as well as *Pseudomonas* sp. Thermostable Kinase 4 (TK4)) had a Ca-terminal extension of 186 amino acids, which was not present in other bacterial sphingomyelinases, as shown in [Table/Fig-2]. As a distinct cluster material, the four Leptospiral sphingomyelinases were clustered by phylogenetic analysis. In Sph1 (597 aa), Sph2 (638 aa), Sph3 (558 aa), and SphH (554 aa), the entire exo-endo-phosphatase domain is present deletions in Sph4 (239 aa); the amino acids in the catalytic site (arrows), the hydrophobic loop (yellow), the solvent exposed loop (green], the P-loop (pink, with a double-headed arrow), and the-hairpin region (red) are all affected [21].



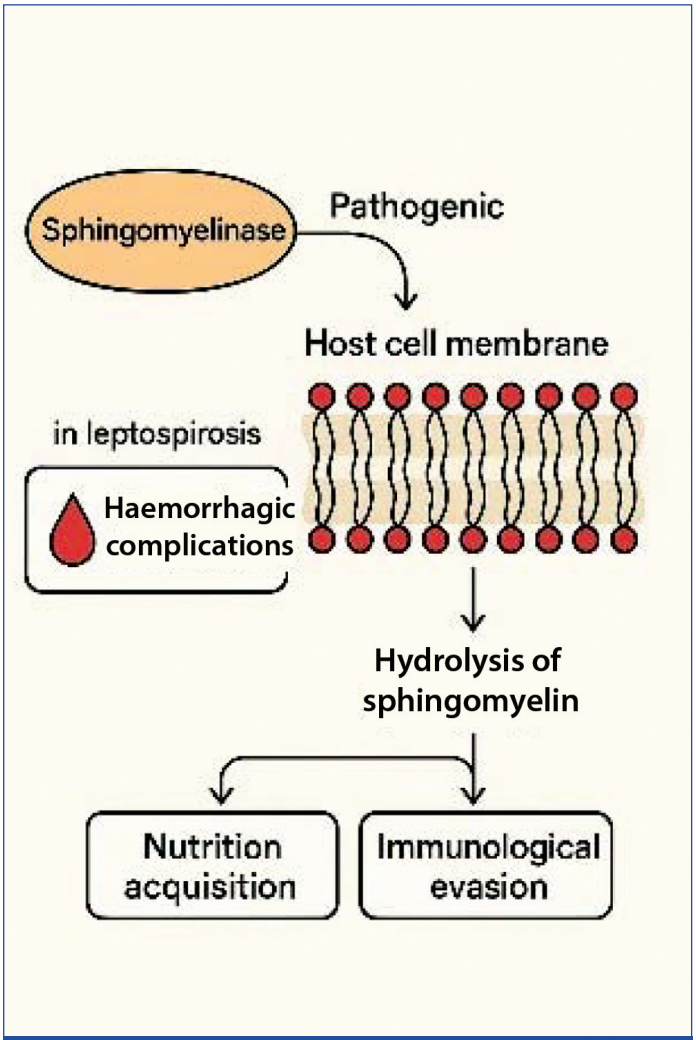
[Table/Fig-1]: Predicted three-dimensional ribbon structure of Leptospiral Sph2 with ceramide.

[Table/Fig-2]: *L. interrogans* serovar Lai Sph2 3D-structure.

Role of Sph2 in Pathogenesis

A potential virulence factor that is only produced by pathogenic *Leptospira* serovars is Leptospiral sphingomyelinase haemolysin. By facilitating cell lysis and obtaining vital nutrients, it is necessary for the pathogenic Leptospires' survival inside the host. Sphingomyelinases also harm host tissues, especially the liver, kidneys, and lungs, which results in bleeding, jaundice, and renal failure [18,22]. Sphingomyelinases are likely released from leptospiral cells via type I or type II secretion pathways [21]. Sph2, a key virulence factor, causes host cell apoptosis and inflammatory tissue damage and serves as a diagnostic marker due to its early presence in infections [23]. Sphingomyelinases are one of these pathogenic agents that are linked to haemorrhagic complications in leptospirosis because they catalyse the hydrolysis of sphingomyelin in host cell membranes. Additionally, these compounds may play a role in nutrition acquisition and immunological evasion [24]. During in-vitro growth, a variety of *Leptospira* strains release sphingomyelinases and haemolysins [25]. The culture supernatant fluid of one Pomona strain co-purified with sphingomyelinase C activity showed a single peak of haemolytic activity by isoelectric focusing, indicating that sphingomyelinase is the cause of haemolysis [26]. Among the

leptospiral sphingomyelinase paralogs, Sph2 has been experimentally demonstrated to possess well-characterised sphingomyelinase C activity, cleaving sphingomyelin into phosphocholine and ceramide [21]. Although Sph1, Sph3, and Sph4 have also been reported to exhibit sphingomyelinase-like activity, their enzymatic properties and biological roles remain less well characterised than those of Sph2. [27]. The non pathogenic *Leptospira biflexa* lacks the genes that code for the sphingomyelinase-like proteins. Likewise, only pathogenic strains of *Leptospira* have been found to exhibit sphingomyelinase activity [28]. According to these findings, sphingomyelinase-like proteins are active during infection [Table/Fig-3] [18].



[Table/Fig-3]: Pathogenic roles of leptospiral sphingomyelinases in haemorrhagic manifestations.

Leptospira-specific sphingomyelinases, which are only found in pathogenic *Leptospira* species, are frequently secreted in the urine of leptospirosis patients within a few days of the infection beginning. The structural and functional significance of Leptospiral sphingomyelinase Sph2 in the pathophysiology of leptospirosis is discussed here, along with the possibility of using urinary Sph2 screening for diagnosis and the possibility of creating a quick and reasonably priced point-of-care test for urinary leptospiral sphingomyelinase Sph2 as a substitute for existing diagnostic techniques [29].

Sph2 as a Diagnostic Marker

Leptospirosis is significantly underdiagnosed due to its diverse symptoms, which range from mild febrile sickness to serious haemorrhage. Since leptospirosis's clinical symptoms are so similar to those of other feverish illnesses, laboratory testing is a crucial and effective method of diagnosing the illness. The current diagnostic methods are laborious, necessitate specialised knowledge and advanced equipment, and are unable to detect the illness in its early stages of infection. Given the serious consequences following infection and the mortality rate following misdiagnosis, early detection of leptospirosis is essential [30]. Sph2, a key virulence factor, induces inflammatory tissue damage and host cell death. Because of its early onset in infections, it also acts as a diagnostic marker [31]. In addition to its diagnostic use, the high antigenicity of Sph2 could be harnessed in the development of a vaccine or treatment [31], however, more studies need to be done to standardise methods used in detection and confirm the reliability of the antigen in different Leptospira serovars.

CONCLUSION(S)

Sph2 has emerged as an important virulence factor and a promising biomarker that can greatly improve the early diagnosis of leptospirosis. Its appearance in the early stages of the infection, as well as its existence in serum and urine, makes it an important biomarker that can be used to detect the disease at the early stages before seroconversion. Its usefulness in endemic co-infection areas is supported by elevated urinary Sph2 levels in patients with dengue-leptospirosis co-infection. It has been shown that diagnostic assays with the incorporation of Sph2 show better accuracy, sensitivity and reliability than the standard diagnostic techniques and thus have made clinical decision-making faster and more reliable. Introduction of Sph2 into easily accessible, point-of-care systems (i.e., rapid immunoassay kits or biosensor-based systems) would make it easier to access early diagnosis in resource-constrained healthcare settings. On the whole, the use of Sph2-based diagnostic approaches can be seen as an important step in the faster, more accurate, and more affordable detection of leptospirosis, which will eventually result in better patient outcomes and allow managing outbreaks in endemic areas.

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