ISSN: 2536-7153 SJMLS

Sokoto Journal of Medical Laboratory Science 2022; 7(2): 31 - 39

SJMLS - 7(2) - 004

Potential Differential Marker in the Diagnosis of Leptospirosis

Shuaibu Abdullahi Hudu[1*] and Mohammed Ibrahim Saeed[2]

Department of Medical Microbiology and Parasitology, Faculty of Basic Clinical Sciences, College of Health Sciences, Usmanu Danfodiyo University, Sokoto, 840232 Sokoto State [1], Department of Microbiology, Faculty of Medical Laboratory Sciences, National Ribat University, Burri 055, Khartoum, Sudan [2]

Author for Correspondence*: hudu.shuaibu@udusok.edu.ng/+234-809-099-312/ORCID: 0000-0001-5765-4580.

https://dx.doi.org/10.4314/sokjmls.v7i2.4

Abstract

Human leptospirosis, or commonly known as the "rat urine disease" is a zoonotic disease caused by a bacterium called *Leptospira sp.* acquired via the urine of animal carriers. It is believed that the incidence rate of leptospirosis has been under-reported due to its unspecific clinical symptoms and the limitations of current diagnostic methods. Leptospirosis can be effectively treated with antibiotics in the early stage, and it is a curable disease. But the accuracy to diagnose the infection is rarely achieved. The present study investigated the plasma protein profiles of leptospirosis patients who presented with different clinical presentations, and compared them against two control groups consisting of dengue patients and healthy individuals. The plasma protein digests were analyzed using a shotgun approach by liquid chromatography-tandem mass spectrometry (LC-MS/MS) as described by the manufacturer. The proteins detected in every leptospirosis patient and with at least two-fold differential expression with statistical significance ($p<0.05$) compared to the control groups were identified. Lipopolysaccharide (LPS)-binding protein (LBP) is reported as the only plasma protein that fulfilled the selection criteria. The characteristics and roles of LBP in the immune system during infections are reviewed. Compared to the other proteome-based studies on human leptospirosis. The present study is the first that reported LBP as one of the significant differentially expressed proteins. Potential of coupling LBP with other disease-specific biomarkers in clinical diagnosis and prognosis should be considered.

Keywords: zoonotic, leptospirosis, spectrometry, chromatography

Introduction

Endemic leptospirosis is a disease with significant health impact in many parts of the world, particularly in the Americas and Asia (World Health Organization, 2006). Clinical presentation of leptospirosis is often non-specific and known to mimic the clinical profile of other prevalent febrile illnesses, such as dengue, influenza and malaria. Clinical manifestations of leptospirosis are ranging from a mild course, such as fever, headache, myalgias, conjunctival suffusion, and abdominal pain; to severe complications characterized by organ failure and bleeding which may lead to death. Different clinical course experienced by different patients have complicated the diagnosis. Misdiagnosis and delayed diagnosis of leptospirosis does usually occur as the accuracy to diagnose the infection are rarely achieved due to the broad spectrum of symptoms and the limitations of the available tests, which may result in inaccurate treatment and poor outcomes. Currently, diagnosis of leptospirosis is performed through the detection of IgM antibodies against the pathogen by microscopic agglutination test (MAT), which is time-consuming and insensitive in early acute-phase specimens due to late seroconversion.

It is hypothesized that the perturbations in the host proteome profile can differentiate not only between individuals with leptospirosis and other clinical resemblance diseases but also between leptospirosis patients with different disease

severity. Srivastava *et al.* (2012) reported the serum proteome of leptospirosis patients showed differentially expressed proteins compared to malaria patients, among those were α-1-antitrypsin, vitronectin, ceruloplasmin, G-protein signalling regulator, and apolipoprotein A-IV. A more recent serum proteome-based study (Ting *et al.*, 2017) demonstrated that apolipoprotein A-I, serum amyloid A, transferrin, haptoglobin, and transthyretin have significant differential expression between mild versus severe leptospirosis patients. In the present pilot study, we aimed to compare the plasma proteome profiles of leptospirosis and dengue patients because of their overlapping geographic distributions and undistinguishable clinical presentations.

Materials and Methods

Informed consent

The study protocol was approved by the Ethical committee of Specialist Hospital Sokoto. Written informed consent was obtained from all included subjects before the sample and data collection.

Enrolment and sample collection

Enrolment of subjects included all clinically suspected leptospirosis patients seen at Outpatient Department of Specialist Hospital, Sokoto from January to June 2021. As for the exclusion criteria, patients in the paediatric age group (below 18 years old) and patients with a history of autoimmune diseases or any known comorbidities were excluded. Healthy volunteers were recruited as the control group. The blood sample was collected in ethylenediaminetetraacetic acid (EDTA) blood vacutainer for obtaining plasma, and in the serum-separating tube for obtaining serum. The plasma/serum was separated by centrifugation at 1,300 *g*, 15 min, 4 °C. The exactly similar collection, processing and storage conditions were maintained for all the samples from the subjects and control participants to minimize any pre-analytical variations.

Plasma sample preparation for mass spectrometry

Plasma protein was estimated by the colorimetric Bradford method using Bio-Rad Protein Assay (Bio-Rad, USA). For each sample, 150-200 g of protein was reduced with 2 mM tris(2-carboxyethyl) phosphine (TCEP; Sigma-Aldrich, USA) at 56 °C for 60 min and alkylated with 20 mM methyl methanethiosulfonate (MMTS; Sigma-Aldrich, USA) at room temperature for 30 minutes. Then, a sequencing grade modified trypsin (Promega, USA) was added to the sample at a trypsin: protein ratio of 1:100 and incubated at 37 °C for 16 hr. Complete digestion was confirmed by comparing an aliquot of the sample before and after the tryptic digestion on a one-dimensional SDS-PAGE. The digested peptides were cleaned up using a strong cation-exchange (SCX) cartridge system (AB SCIEX, USA) and the SCX eluent was desalted using Pierce C18 tips (Thermo Scientific, USA).

Liquid chromatography-tandem mass spectrometry

The protein identification was performed using the shotgun approach by liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. In brief, the desalted eluent was adjusted to about 1.0 g/L before being subjected to the chip LCD-nanoflex system (Eksigent, USA) coupled to a TripleTOF 5600 system (AB SCIEX, USA). The tandem mass spectra of peptides were then extracted from raw files and searched against the Swiss-Prot protein database (released on 16 February 2017, 553,474 sequences) using MASCOT Server 2.4.0 (Matrix Science, UK). The cut-off threshold for protein identification was set at a 1% false discovery rate (FDR).

Statistical analysis

Only proteins detected in all the three leptospirosis patients and with at least two-fold differential expression compared to the dengue and healthy control groups were selected. The difference of protein content was compared statistically with independent samples t-test using SPSS Statistics v25 (IBM, USA) and those with statistical significance $p<0.05$ were identified.

Results

Patient clinical characteristics

With the consideration of inclusion criteria as well as age- and gender- matching, three of leptospirosis (denoted as LEPTO-1, LEPTO-2, LEPTO-3; mean age 25.7) and one of dengue (denoted as DEN-CTRL; age 24) male patients; together with two

healthy males (denoted as HLTY-CTRL1, HLTY-CTRL2; mean age 28) were selected for proteomic profiling and comparison.

Upon admission, the three leptospirosis and the one dengue patients were presented with some common clinical manifestations, including fever, gastrointestinal symptoms, and myalgia. None of the leptospirosis patients was tested positive in dengue detection as well as in latex agglutination test for leptospirosis. The LEPTO-1 patient had developed immunity against leptospirosis (MAT titer 1:400) as early as day-3 since the disease symptom onset. Despite the highest level of C-reactive protein and creatine kinase as well as high creatinine recorded in the LEPTO-1 among the three leptospirosis patients, his clinical symptoms were quickly resolved after being treated accordingly. Similarly, the LEPTO-2 patient was also gradually recovered throughout hospitalization. However, his diagnosis for leptospirosis was not confirmed until comparing the paired sera that were collected from him upon admission and discharge. In contrast, the LEPTO-3 patient was admitted to the hospital due to persisted fever and diarrhoea for two weeks and presented with mild jaundice, shortness of breath, melena, hepatorenal syndrome, and severe metabolic acidosis. His chest x-ray showed minimal bilateral pleural effusions. Detection of IgM against *Leptospira* in his blood upon admission was confirmed by MAT. He was then being cared for in the intensive care unit and eventually discharged after recovery. Lastly, for the DEN-CTRL, his paired sera tested by MAT did not conclude a leptospirosis infection.

Differentially expressed proteins

The proteins detected in all the three leptospirosis patients and with at least two-fold differential expression compared to the dengue or/and healthy control groups are shown in Table 1. Lipopolysaccharide (LPS)-binding protein (LBP) is the only plasma protein that met all the selection criteria. On the other hand, the protein contents of haptoglobin, C-reactive protein, leucine-rich alpha-2-glycoprotein, and alpha-1-antichymotrypsin are more than two-fold compared to the healthy control group yet are much lower than two-fold difference in compared to the DEN-CTRL, which is unable to distinguish leptospirosis from dengue fever.

Table 1. Expression of Leptospira proteins in case and control groups

NAME OF PROTEIN	FOLD CHANGE	
	LEPTO vs CTRL-1	LEPTO vs CTRL-2
C-reactive protein	0.18	3.72
Lipopolysaccharide binding protein	7.20	14.63
Alpha -1 anti -chymotrypsin	0.83	2.33
Alpha -2 glycoprotein	0.79	3.98
Haptoglobin	0.72	4.45

LEPTO: protein contents of leptospirosis; **CTR**L: Protein contents of control group

Discussion

Infection with pathogenic *Leptospira* species causes leptospirosis. *Leptospira* is a genus of Gram-negative spirochaete bacteria that historically being distributed into two species: *L. interrogans* comprised of the pathogenic serovars, and *L. biflexa* comprised of the saprophytic serovars (Faine and Stallman, 1982). More than 250 *Leptospira* serovars have been reported based on the heterogeneity in the carbohydrate component of LPS, and those with shared antigenicity are clustered into serogroups (Levett, 2001). The serovar identity is attributed to the differences in O-antigen polysaccharide of LPS (Bulach *et al.*, 2000), which is determined by MAT that uses a panel of live leptospires

representing different serogroups as antigens for detection of agglutinating antibodies (Turner, 1968). The antigen-antibody reactions of MAT assay may not be used to predict the identity of the causative serovars (Levett, 2003; Smythe *et al.*, 2009). Direct isolation and molecular identification are the more appropriate methods for identifying precisely the infecting serovars. Alongside the serological classification scheme, *Leptospira* species are also classified based on genetic relatedness, initially by DNA-DNA hybridization (Brenner *et al.*, 1999; Ramadass *et al.*, 1992; Yasuda *et al.*, 1987) and latterly supported by 16s rRNA phylogenetic inference (Morey *et al.*, 2006). However, the two classification systems do not always coincide (Levett, 2001). Even though the genotypic classification system is taxonomically legitimate, the serological classification provides epidemiological data for clinicians and microbiologists. The co-existence of two classification systems for *Leptospira* is necessary to serve both evolutionary and epidemiology purposes.

Besides the role in serological classification, leptospiral LPS serves as the principal antigen recognized by the human immune system during *Leptospira* infections. This is in complete contrast to other major disease-causing spirochetes that have no LPS, including *Borrelia burgdorferi* (Fraser *et al.*, 1997) that causes Lyme disease; and *Treponema pallidum* (Fraser *et al.*, 1998) that causing syphilis. Leptospiral LPS has been proven antigenically active in mice, causing a higher endotoxic effect to liver necrosis but lower lethality compared with *Escherichia coli* LPS (Isogai *et al.*, 1986). Administration of monoclonal antibodies directed against LPS determinants in an animal model confers protection against the lethality of leptospirosis (Jost *et al.*, 1986). Leptospiral LPS has a generally lower endotoxicity compared with Gram-negative LPS, possibly related to its atypical lipid A component (Que-Gewirth *et al.*, 2004).

LBP, a soluble positive acute-phase protein is playing a central role in the early step of host defence to Gram-negative bacterial infections or LPS. It is a 50-kDa polypeptide that is mainly synthesized in hepatocytes (Ramadori *et al.*, 1990) and some are found to be produced by lung (Dentener *et al.*, 2000) and intestinal (Vreugdenhil *et al.*, 2000) epithelial cells. It is then released as a ~60-kDa glycoprotein after glycosylation (Tobias *et al.*, 1986). The level of LBP in normal serum is about 5-10 g/mL, and may elevate to above 200 g/mL in acute-phase serum (Tobias *et al.*, 1992). Lamping *et al.* (1998) proposed a concentration-dependent dual role of LBP in the pathogenesis of Gram-negative bacterial sepsis. Low concentrations of LBP enhanced LPS-induced tumour necrosis factor-alpha (TNF-α) synthesis whereas acute phase concentrations of LBP inhibited this effect. High concentrations of LBP have been shown to reduce LPS activity (Zweigner *et al.*, 2001), and to neutralize LPS (Gutsmann *et al.*, 2001; Hailman *et al.*, 1996; Wurfel *et al.*, 1994), as well as to result in better disease outcome (Opal *et al.*, 1999). Collectively, high LBP concentration in the host during sepsis has been described as a protective function from uncontrolled inflammatory responses.

LBP opsonizes the LPS (Wright *et al.*, 1989) by recognizing its lipid A moiety to form LBP-LPS complexes (Tobias *et al.*, 1989) and presents the LPS to important pattern recognition receptors to initiate the innate immune system. LBP-LPS complexes activate macrophages/monocytes and neutrophils for phagocytosis by binding to their membrane-bound CD14 (mCD14) (Hailman *et al.*, 1994; Wright *et al.*, 1990). LBP may also catalyze LPS binding with the soluble CD14 (sCD14) to activate those mCD14-negative endothelial and epithelial cells (Pugin *et al.*, 1993) for cytokine release. A single LBP may transport up to 500 LPS molecules to sCD14 yet LBP is not consumed in such catalysis (Tobias *et al.*, 1995). In addition, LBP appears capable of transferring LPS to high-density lipoproteins (HDL) and leading to LPS neutralization (Hamann *et al.*, 2005; Wurfel *et al.*, 1994).

Muta and Takeshige (2001) suggested the interactions of CD14 with LBP is necessary to activate toll-like receptors 2 and 4 (TLR2 and TLR4) signaling cascade in response to LPS. TLR4 is known as the predominant receptor of the innate immune system that recognizes Gram-

negative LPS (Tapping *et al.*, 2000; Poltorak *et al.*, 1998), which is in contrast to TLR2 that recognizes Gram-positive bacteria (Schwandner *et al.*, 1999; Yoshimura *et al.*, 1999) and other non-LPS products (Aliprantis *et al.*, 1999; Lien *et al.*, 1999). Leptospiral LPS can activate both TLR2 and TLR4 in murine cells (Nahori *et al.*, 2005) but activates only the TLR2 pathway in human cells (Werts *et al.*, 2001). The efficient detection of LPS by both the TLR2 and TLR4 signaling cascades may have to render the mice resistant to leptospirosis.

As the name implies, LBP is previously thought to bind and respond only to Gram-negative LPS when it was first characterized (Tobias *et al.*, 1986). However, many studies revealed that the LBP involvement in immune responses does not limit to against LPS in Gram-negative infections. It has been proven to interact with other components, including lipoteichoic acid (LTA) and peptidoglycans of Gram-positive bacteria (Fan *et al.*, 1999;), LTA-like lipoglycans of spirochaetes (Schröder *et al.*, 2001), glycolipids (Schröder *et al.*, 2003; Weber *et al.*, 2003; Vignal *et al.*, 2003; Schröder *et al.*, 2000), and lipoproteins (Schröder *et al.*, 2004; Vreugdenhil *et al.*, 2003). Several clinical studies have reported elevated LBP in patients with Gram-negative, Gram-positive, and fungal infections as well as those with other medical conditions (Blairon *et al.*, 2003; Zweigner *et al.*, 2001; Opal *et al.*, 1999). The utility of LBP as a single diagnostic biomarker for any diseases or leptospirosis might not be clinically practical and should be evaluated carefully. Nevertheless, the potential of coupling LBP with other disease-specific biomarkers in clinical diagnosis and prognosis should be considered.

Compared to the other proteome-based studies on human leptospirosis, the present study is the first that reported LBP as one of the significant differentially expressed proteins, which is not noted in the studies of Srivastava *et al.* (2012) as well as Ting *et al.* (2017). One explanation could be that different study designs and control groups were used in the protein profile comparisons. Srivastava *et al.* (2012) compared the serum proteome profile of leptospirosis against both malaria patients and healthy volunteers whereas Ting *et al.* (2017) compared mild versus severe leptospirosis against healthy individuals. In considering that additional sample pretreatments before the mass spectrometry analysis, including albumin/IgG depletion and protein separation by two-dimensional gel electrophoresis (2-DE), loss of LBP during the laboratory procedures or masking of LBP by other highly abundant proteins on the 2-DE gels cannot be ruled out.

Conclusion

The results obtained from this small number of patients may not represent the clinical course of leptospirosis in general. In addition to the need for a larger cohort size with different degrees of clinical manifestations, the inclusion of other febrile diseases as control groups for verification may be required. Instead of using LBP as a single diagnostic biomarker, a biomarker panel comprises one or more host- and pathogen-derived proteins should be developed.

References

Aliprantis, A.O., Yang, R.B., Mark, M.R., Suggett, S., Devaux, B., Radolf, J.D., Klimpel, G.R., Godowski, P., and Zychlinsky, A. (1999). Cell activation and apoptosis by bacterial lipoproteins through toll-like receptor-2. *Science;* ***285***: 736-739.

Blairon, L., Wittebole, X., and Laterre, P.F. (2003). Lipopolysaccharide-binding protein serum levels in patients with severe sepsis due to Gram-positive and fungal infections. *The Journal of Infectious Diseases;* ***187***: 287-291.

Brenner, D.J., Kaufmann, A.F., Sulzer, K.R., Steigerwalt, A.G., Rogers, F.C., and Weyant, R.S. (1999). Further determination of DNA relatedness between serogroups and serovars in the family *Leptospiraceae* with a proposal for *Leptospira alexanderi* sp. nov. and four new *Leptospira* genomospecies. *International Journal of Systemic Bacteriology;* ***49***: 839-858.

Bulach, D.M., Kalambaheti, T., Pena-Moctezuma, A., and Adler, B. (2000). Lipopolysaccharide biosynthesis in *Leptospira*. *Journal of Molecular Microbiology and Biotechnolology;* **2(4)**:375-380.

Dentener, M.A., Vreugdenhil, A.C.E., Hoet,

P.H.M., Vernooy, J.H.J., Nieman, F.H.M., Heumann, D., Janssen, Y.M.W., Buurman, W.A., and Wouters, E.F.M. (2000). Production of the acute-phase protein lipopolysaccharide-binding protein by respiratory type II epithelial cells. *American Journal of Respiratory Cell Molecular Biology; **23**:* 146-153.

Faine, S., and Stallman, N.D. (1982). Amended descriptions of the genus *Leptospira* Noguchi 1917 and the species *L. interrogans* (Stimson 1907) Wenyon 1926 and *L. biflexa* (Wolbach and Binger 1914) Noguchi 1918. *International Journal of Systematic Evolution Microbiology; **32**:* 461-463.

Fan, X., Stelter, F., Menzel, R., Jack, R., Spreitzer, I., Hartung, T., and Schütt, C. (1999). Structures in *Bacillus subtilis* are recognized by CD14 in a lipopolysaccharide binding protein-dependent reaction. *Infection Immunology; **67**:* 2964-2968.

Fraser, C.M., Casjens, S., Huang, W.M., Sutton, G.G., Clayton, R., Lathigra, R., White, O., Ketchum, K.A., Dodson, R., Hickey, E.K.*, et al.* (1997). Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi*. *Nature; **390**:* 580-586.

Fraser, C.M., Norris, S.J., Weinstock, G.M., White, O., Sutton, G.G., Dodson, R., Gwinn, M., Hickey, E.K., Clayton, R., Ketchum, K.A.*, et al.* (1998). Complete genome sequence of *Treponema pallidum*, the syphilis spirochete. *Science; **281**:* 375-388.

Gutsmann, T., Müller, M., Carroll, S.F., MacKenzie, R.C., Wiese, A., and Seydel, U. (2001). Dual role of lipopolysaccharide (LPS)-binding protein in neutralization of LPS and enhancement of LPS-induced activation of mononuclear cells. *Infection and Immunology; 69:* 6942-6950.

Hailman, E., Albers, J.J., Wolfbauer, G., Tu, A.-Y., and Wright, S.D. (1996). Neutralization and transfer of lipopolysaccharide by phospholipid transfer protein. *Journal of Biology and Chemistry; **271**: 1*2172-12178.

Hailman, E., Lichenstein, H.S., Wurfel, M.M., Miller, D.S., Johnson, D.A., Kelley, M., Busse, L.A., Zukowski, M.M., and Wright, S.D. (1994). Lipopolysaccharide (LPS)-binding protein accelerates the binding of LPS to CD14. *The Journal of Experimental Medicine; **179**:* 269-277.

Hamann, L., Alexander, C., Stamme, C., Zähringer, U., and Schumann, R.R. (2005). Acute-phase concentrations of lipopolysaccharide (LPS)-binding protein inhibit innate immune cell activation by different LPS chemotypes via different mechanisms. Infection and Immunology; *73:* 193-200.

Isogai, E., Isogai, H., Kurebayashi, Y., and Ito, N. (1986). Biological activities of leptospiral lipopolysaccharide. Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene Series A: Medical Microbiology, Infectious Diseases, Virology, *Parasitology; **261**:* 53-64.

Jost, B.H., Adler, B., Vinh, T., and Faine, S. (1986). A monoclonal antibody reacting with a determinant on leptospiral lipopolysaccharide protects guinea pigs against leptospirosis. *Journal of Medical Microbiology; **22**:* 269-275.

Lamping, N., Dettmer, R., Schröder, N.W., Pfeil, D., Hallatschek, W., Burger, R., and Schumann, R.R. (1998). LPS-binding protein protects mice from septic shock caused by LPS or gram-negative bacteria. *The Journal of Clinical Investigation; **101**:* 2065-2071.

Levett, P.N. (2001). Leptospirosis. *Clinical Microbiology Review; **14**:* 296-326.

Levett, P.N. (2003). Usefulness of serologic analysis as a predictor of the infecting serovar in patients with severe leptospirosis. *Clinical Infectios Disease; **36**:* 447-452.

Lien, E., Sellati, T.J., Yoshimura, A., Flo, T.H., Rawadi, G., Finberg, R.W., Carroll, J.D., Espevik, T., Ingalls, R.R., Radolf, J.D.*, et al.* (1999). Toll-like receptor 2 functions as a pattern recognition receptor for diverse bacterial products. *Journal of Biology and Chemistry; **274**:* 33419-33425.

Morey, R.E., Galloway, R.L., Bragg, S.L., Steigerwalt, A.G., Mayer, L.W., and Levett, P.N. (2006). Species-specific identification of *Leptospiraceae* by 16S rRNA gene sequencing. *Journal of Clinical Microbiology; **44**:* 3510-3516.

Muta, T., and Takeshige, K. (2001). Essential roles of CD14 and lipopolysaccharide-binding protein for activation of toll-like

receptor (TLR)2 as well as TLR4. *European Journal of Biochemistry; **268**:* 4580-4589.

Nahori, M.-A., Fournié-Amazouz, E., Que-Gewirth, N.S., Balloy, V., Chignard, M., Raetz, C.R.H., Saint Girons, I., and Werts, C. (2005). Differential TLR recognition of leptospiral lipid A and lipopolysaccharide in murine and human cells. *The Journal of Immunology; **175**:* 6022-6031.

Opal, S.M., Scannon, P.J., Vincent, J.-L., White, M., Carroll, S.F., Palardy, J.E., Parejo, N.A., Pribble, J.P., and Lemke, J.H. (1999). Relationship between plasma levels of lipopolysaccharide (LPS) and LPS-binding protein in patients with severe sepsis and septic shock. *The Journal of Infectious Diseases; **180**:* 1584-1589.

Poltorak, A., He, X., Smirnova, I., Liu, M.-Y., Huffel, C.V., Du, X., Birdwell, D., Alejos, E., Silva, M., Galanos, C., *et al.* (1998). Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in *Tlr4* gene. *Science; **282**:* 2085-2088.

Pugin, J., Schürer-Maly, C.C., Leturcq, D., Moriarty, A., Ulevitch, R.J., and Tobias, P.S. (1993). Lipopolysaccharide activation of human endothelial and epithelial cells is mediated by lipopolysaccharide-binding protein and soluble CD14. *Proceeding of the National Academy of Science USA; **90**:* 2744-2748.

Que-Gewirth, N.L.S., Ribeiro, A.A., Kalb, S.R., Cotter, R.J., Bulach, D.M., Adler, B., Girons, I.S., Werts, C., and Raetz, C.R.H. (2004). A methylated phosphate group and four amide-linked acyl chains in *Leptospira interrogans* lipid A: the membrane anchor of an unusual lipopolysaccharide that activates TLR2. *Journal of Biology and Chemistry; **279**:* 25420-25429.

Ramadass, P., Jarvis, B.D.W., Corner, R.J., Penny, D., and Marshall, R.B. (1992). Genetic characterization of pathogenic *Leptospira* species by DNA hybridization. *International Journal of Systematic Bacteriology; **42**:* 215-219.

Ramadori, G., Meyer zum Buschenfelde, K.H., Tobias, P.S., Mathison, J.C., and Ulevitch, R.J. (1990). Biosynthesis of lipopolysaccharide-binding protein in rabbit hepatocytes. *Pathobiology; **58**:* 89-94.

Schröder, N.W.J., Heine, H., Alexander, C., Manukyan, M., Eckert, J., Hamann, L., Göbel, U.B., and Schumann, R.R. (2004). Lipopolysaccharide binding protein binds to triacylated and diacylated lipopeptides and mediates innate immune responses. The *Journal of Immunology; **173**:* 2683-2691.

Schröder, N.W.J., Morath, S., Alexander, C., Hamann, L., Hartung, T., Zähringer, U., Göbel, U.B., Weber, J.R., and Schumann, R.R. (2003). Lipoteichoic acid (LTA) of *Streptococcus pneumoniae* and *Staphylococcus aureus* activates immune cells via toll-like receptor (TLR)-2, lipopolysaccharide-binding protein (LBP), and CD14, whereas TLR-4 and MD-2 are not involved. *Journal of Biology and Chemistry; **278**:* 15587-15594.

Schröder, N.W.J., Opitz, B., Lamping, N., Michelsen, K.S., Zähringer, U., Göbel, U.B., and Schumann, R.R. (2000). Involvement of lipopolysaccharide binding protein, CD14, and toll-like receptors in the initiation of innate immune responses by *Treponema* glycolipids. *The Journal of Immunology; **165**:* 2683-2693.

Schröder, N.W.J., Pfeil, D., Opitz, B., Michelsen, K.S., Amberger, J., Zähringer, U., Göbel, U.B., and Schumann, R.R. (2001). Activation of mitogen-activated protein kinases p42/44, p38, and stress-activated protein kinases in myelo-monocytic cells by*Treponema* lipoteichoic acid. *Journal of Biology and Chemistry;**276**:* 9713-9719.

Schwandner, R., Dziarski, R., Wesche, H., Rothe, M., and Kirschning, C.J. (1999). Peptidoglycan- and lipoteichoic acid-induced cell activation is mediated by toll-like receptor 2. *Journal of Biology and Chemistry; **274**:* 17406-17409.

Smythe, L.D., Wuthiekanun, V., Chierakul, W., Suputtamongkol, Y., Tiengrim, S., Dohnt, M.F., Symonds, M.L., Slack, A.T., Apiwattanaporn, A., Chueasuwanchai, S., *et al.* (2009). The microscopic agglutination test (MAT) is an unreliable predictor of infecting *Leptospira* serovar in Thailand. *American Journal of Tropical Medicine and Hygiene;**81**:* 695-697.

Srivastava, R., Ray, S., Vaibhav, V., Gollapalli, K., Jhaveri, T., Taur, S., Dhali, S., Gogtay, N.,

Thatte, U., Srikanth, R., *et al.* (2012). Serum profiling of leptospirosis patients to investigate proteomic alterations. *Journal of Proteomics; **76**:* 56-68.

Tapping, R.I., Akashi, S., Miyake, K., Godowski, P.J., and Tobias, P.S. (2000). Toll-like receptor 4, but not toll-like receptor 2, is a signaling receptor for *Escherichia* and *Salmonella* lipopolysaccharides. *The Journal of Immunology; **165**:* 5780-5787.

Ting, T.X., Amran, F.b., Thayan, R., Ahmad, N., Jaafar, R., Haron, R., Abdullah, R., bin Shamsuddin, S.R., Md. Riffin, N.S.b., and Abdul-Rahman, P.S. (2017). Potential serum biomarkers associated with mild and severe leptospirosis infection: A cohort study in the Malaysian population. *Electrophoresis; **38**:* 2141-2149.

Tobias, P.S., Mathison, J., Mintz, D., Lee, J.-D., Kravchenko, V., Kato, K., Pugin, J., and Ulevitch, R.J. (1992). Participation of lipopolysaccharide-binding protein in lipopolysaccharide-dependent macrophage activation. *American Journal of Respiratory Cell Molecular Biology; 7:* 239-245.

Tobias, P.S., Soldau, K., Gegner, J.A., Mintz, D., and Ulevitch, R.J. (1995). Lipopolysaccharide binding protein-mediated complexation of lipopolysaccharide with soluble CD14. *Journal of Biology and Chemistry; **270***: 10482-10488.

Tobias, P.S., Soldau, K., and Ulevitch, R.J. (1986). Isolation of a lipopolysaccharide-binding acute phase reactant from rabbit serum. *The Journal of Experimental Medicine; **164**:* 777-793.

Tobias, P.S., Soldau, K., and Ulevitch, R.J. (1989). Identification of a lipid A binding site in the acute phase reactant lipopolysaccharide binding protein. *Journal of Biology and Chemistry; **264**:* 10867-10871.

Turner, L.H. (1968). Leptospirosis II: serology. *Translation of Royal Society of Tropical Medicine and Hygiene; **62**:* 880-899.

Vignal, C., Guérardel, Y., Kremer, L., Masson, M., Legrand, D., Mazurier, J., and Elass, E. (2003). Lipomannans, but not lipoarabinomannans, purified from *Mycobacterium chelonae* and *Mycobacterium kansasii* induce TNF-α and IL-8 secretion by a CD14-toll-like receptor 2-dependent mechanism. *The Journal of Immunology; **171**:* 2014-2023.

Vreugdenhil, A.C.E., Rousseau, C.H., Hartung, T., Greve, J.W.M., van 't Veer, C., and Buurman, W.A. (2003). Lipopolysaccharide (LPS)-binding protein mediates LPS detoxification by chylomicrons. *The Journal of Immunology; **170**:* 1399-1405.

Vreugdenhil, A.C.E., Snoek, A.M.P., Greve, J.W.M., and Buurman, W.A. (2000). Lipopolysaccharide-binding protein is vectorially secreted and transported by cultured intestinal epithelial cells and is present in the intestinal mucus of mice. *The Journal of Immunology; 165:* 4561-4566.

Weber, J.R., Freyer, D., Alexander, C., Schröder, N.W.J., Reiss, A., Küster, C., Pfeil, D., Tuomanen, E.I., and Schumann, R.R. (2003). Recognition of pneumococcal peptidoglycan: an expanded, pivotal role for LPS binding protein. *Immunity; **19**:* 269-279.

Werts, C., Tapping, R.I., Mathison, J.C., Chuang, T.-H., Kravchenko, V., Saint Girons, I., Haake, D.A., Godowski, P.J., Hayashi, F., Ozinsky, A., *et al.* (2001). Leptospiral lipopolysaccharide activates cells through a TLR2-dependent mechanism. *Nature Immunology; **2**:* 346-352.

World Health Organization, W. (2006). Informal consultation on global burden of leptospirosis: Methods of assessment (Geneva: World Health Organization).

Wright, S.D., Ramos, R.A., Tobias, P.S., Ulevitch, R.J., and Mathison, J.C. (1990). CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. *Science ;**249**:* 1431-1433.

Wright, S.D., Tobias, P.S., Ulevitch, R.J., and Ramos, R.A. (1989). Lipopolysaccharide (LPS) binding protein opsonizes LPS-bearing particles for recognition by a novel receptor on macrophages. *The Journal of Experimental Medicine; **170**:* 1231-1241.

Wurfel, M.M., Kunitake, S.T., Lichenstein, H., Kane, J.P., and Wright, S.D. (1994). Lipopolysaccharide (LPS)-binding protein is carried on lipoproteins and acts as a cofactor in the neutralization of LPS. *The*

Journal of Experimental Medicine; ***180****:* 1025-1035.

Yasuda, P.H., Steigerwalt, A.G., Sulzer, K.R., Kaufmann, A.F., Rogers, F., and Brenner, D.J. (1987). Deoxyribonucleic acid relatedness between serogroups and serovars in the family *Leptospiraceae* with proposals for seven new *Leptospira* species. *International Journal of Systematic Evolution and Microbiology*; ***37****:* 407-415.

Yoshimura, A., Lien, E., Ingalls, R.R., Tuomanen, E., Dziarski, R., and Golenbock, D. (1999). Cutting edge: recognition of Gram-positive bacterial cell wall components by the innate immune system occurs via toll-like receptor 2. *The Journal of Immunology;* ***163***: 1-5.

Zweigner, J., Gramm, H.-J., Singer, O.C., Wegscheider, K., and Schumann, R.R. (2001). High concentrations of lipopolysaccharide-binding protein in serum of patients with severe sepsis or septic shock inhibit the lipopolysaccharide response in human monocytes. *Blood;* ***98****:* 3800-3808.

Citation: Shuaibu Abdullahi Hudu and Mohammed Ibrahim Saeed. Potential Differential Marker in the Diagnosis of Leptospirosis. ***Sokoto Journal of Medical Laboratory Science; 7(2): 31 - 39.*** https://dx.doi.org/10.4314/sokjmls.v7i2.4