Perspectives to DENV and GAS Infections and Associated Serum LDH Profiles

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Abstract

The dengue fever that is caused with the infection of dengue viruses (DENV) progress rapidly into severe forms (DHF and DSS) has been considered a major mosquito-borne health problem worldwide. On the other hand, the group A streptococci (GAS; also called *Streptococcus pyogenes*) causes severe invasive infections including STSS and stands a major health disorder in the globe. An early diagnosis and treatment remain the vital factors in accomplishing successful control of both the devastating diseases from DENV and GAS infection, and therefore, global surveillance of this bacterial pathogen is strongly recommended. This review is, therefore, an epidemiological update of the diseases based on the published data obtained from various scientific websites as well as published research from SCI and non-SCI journals.

Keywords: Dengue Viruses; Group A Streptococci Infection; Dengue Haemorrhagic Fever; Dengue Shock Syndrome; Streptococcal Toxic Shock-Like Syndrome; Serum Lactate Dehydrogenase Profiles

Abbreviation

ARF: Acute Rheumatic Fever; DF: Dengue Fever; DENVs: Dengue Viruses; DHF: Dengue Haemorrhagic Fever; DSS: Dengue Shock Syndrome; EIA: Enzyme Immunoassays; GAS: Group A Streptococci; LA: latex Agglutination; LDH: Lactate Dehydrogenase; LOIAs: liposomal and Optical Immunoassays; PSGN: Post-Infection Streptococcal Glomerulonephritis; RHD: Rheumatic Heart Disease; STSS: Streptococcal Toxic Shock-Like Syndrome; WHO: World Health Organization

Introduction

Humans are time and again exposed to a variety of viral and bacterial pathogens, sometimes the viral infections are complicated by a coexisting, or succeeding, bacterial infection called co-infections or super infections, respectively, and due to combined effects, the mortality of infections is even greater than that of either the virus or the bacteria alone [1]. The WHO ranks the GAS in the top 10 leading causes of morbidity and mortality from infectious diseases worldwide [2]. On the other hand, dengue is now one of the most important neglected tropical diseases, in the globe, and the disease incidence has increased > 30 fold along with the geographical extension of the mosquito vectors as well as DENVs, and thus, dengue epidemics inflict 'high costs to health services, to families and to the economic systems' of affected countries [3,4]. Considering the severity of the diseases, the current review underlines the importance of LDH as a determining factor of the infection with GAS (bacterial pathogenic agent) and DENV (viral pathogenic agent) for prompt diagnosis and proper management of infections, since these are two important pathogens endemic to India [5,6], and have the capacity to cause multiple diseases, some of which are life-threatening, such as, STSS, ARF, PSGN due to GAS infection, and DHF and DSS due to DENV infection. The entire review was based on the evidence documented in SCI as well as non-SCI journals.

Group A streptococci infection

Disease etiology

The *Streptococcus pyogenes,* which is also known as group A Streptococcus (GAS), is a vital human pathogen responsible for causing several diseases with varied clinical manifestations; from non-invasive skin infection and pharyngitis to an array of invasive illnesses, such as bacteraemia, cellulitis and necrotizing fasciitis, which may further be complicated by the development of STSS. The *S. pyogenes* (belonging to Lancefield serogroup A) is a gram-positive β-hemolytic bacterium and is non-motile, non-spore-forming coccus (0.5 - 1.2 μm in size); the bacteria habitually grow in pairs or chains and are negative for oxidase and catalase production [7,8]. The infection with GAS is related to two potentially serious post-infection immune sequelae: ARF and PSGN develop due to repeated GAS exposure, and in addition, infection with the pathogen [7]. The *S. pyogenes* has the capacity to colonize the upper part of the respiratory tract and is highly virulent as it overcomes the host defense system. The invasive GAS disease has been reported to include 'septic arthritis, puerperal sepsis, meningitis, abscess, osteomyelitis, endocarditis and peritonitis' [9]. The bacterium inhabits the throat and skin and is transmitted through inhalation of hefty of droplets from infected patients or through skin to skin contact [10]. The life-threatening infection begins when the bacteria extend below the surface of the skin or throat, and invade the underlying soft tissue, due to the action of an array of virulence factors: M protein (this is surface-anchored protein that forms the basis for the serological demarcation of GAS strains), hyaluronic acid capsule, streptolysin O (SLO; a 69 kDa cholesterol-dependent cytolysin having the capacity to oligomerize forming pores of ~25-30 nm in host cell membranes), streptokinase and plasmin acquisition (streptoknase is the secretion from GAS converting the pro-enzyme plasminogen to plasmin) [7,11].

Disease epidemiology

An estimated 6.63×10^5 cases of invasive GAS disease occur worldwide each year, resulting in 1.63×10^5 deaths [7]. The WHO ranks GAS in the top 10 leading causes of morbidity and mortality from infectious diseases, responsible for over 5×10^5 death cases annually [2]. Mucosal (throat) and epithelial (skin) surfaces represent the GAS primary ecological niches, where GAS causes over 700 million reported cases of purulent, self-limiting infections, such as pharyngitis and impetigo, worldwide each year [12]. GAS can also gain access to normally sterile sites of the body (viz., soft tissue, blood stream) and produce life-threatening invasive diseases (necrotizing fasciitis and STSS) [13,14].

It has been estimated that severe *S. pyogenes* infection lead to 5.17×10^5 deaths per year globally, in addition to 2.33×10^5 deaths caused by rheumatic fever disease [2], while in the United States, 1800 invasive GAS infection-related deaths (necrotizing fasciitis and STSS) have been reported annually [15, 16]. In India, the disease burden due to GAS infection is considerable [5] and the incidence of ARF and rheumatic heart disease ranged from 0.3 to 5.4 per 1,000 children, as has been reported by Padmavati [17]. The GAS infection related pharyngitis has been reported to be highly prevalent in north India [14], while pyoderma has been in frequent in south India [18]. Therefore, the importance of epidemiologic studies on GAS infection in India has direct association with an urgent need of vaccine development.

Culture and Biochemical tests

The culture of throat swab is recognized as the most reliable technique to detect the presence of GAS, and the presumptive identification of the β-hemolytic group A streptococci relies on the susceptibility to bacitracin [9,19]. When cultured on blood agar plates, the production of a characteristic zone of complete haemolysis (β-haemolysis) is another important clue to the classification of *S. pyogenes* [20]. The confirmation of GAS infection has been done serologically following the Lancefield capillary precipitin technique and slide agglutination procedure, which utilize standardized grouping antisera, as has been reported previously [8,19]. The serodiagnosis of *S. pyogenes* infection has been reported to perform on the basis of immune responses against a few extracellular products including 'streptolysin O, DNase B, hyaluronidase, NADase, and streptokinase', which induce strong immune responses in the infected person [8]. The tests such as co-agglutination, LA, EIA and LOIAs have also been employed for GAS identification; most of them have sensitivity between 70% and 90%, but with a specificity of > 95% considering culture as the gold standard technique [21]. If there are outbreaks of GAS sepsis, then it is useful to apply molecular techniques to identify different strains [10,22]. The confirmation of the diagnosis, for GAS infection, by cultural technique, as the accepted reference standard, showed an accuracy of 90 - 97% [23], but the method is slow and not readily available in rural settings of developing countries, like India. Rapid GAS antigen detection tests (RADTs) provides decision within 10 - 15 minutes, with specificity of 90% and sensitivity of > 90% [23,24], and a positive test is accepted for diagnosis of GAS infection, but a negative test is required to be followed by throat culture [24,25], for confirmation.

Treatment of GAS Infection

Once the etiology of *S. pyogenes* is confirmed, high-dose penicillin and clindamycin are prescribed [26]. Either penicillin, or its derivatives: amoxicillin and ampicillin, remain the antibiotics of choice for the treatment of non-allergic patients infected with *S. pyogenes*, while for allergic individuals, azithromycin and clarithromycin are recommended, and for severe *S. pyogenes* infection (necrotizing fasciitis, TSS), penicillin is given in combination with clindamycin [27]. However, clindamycin and vancomycin resistance among S. pyogenes are most concerning [28].

Dengue infection

Dengue virus and the disease etiology

The disease, dengue is caused by the infection DENV, which is a member of the *Flavivirus* genus, within the Flaviviridae family. The four DENV serotypes (DENV1 to DENV4) differ by 25 - 35 base pairs nucleotide sequence, and each of the four serotypes possesses the capacity to cause dengue; of the four serotypes, DENV4 appears to be the most divergent one, followed by DENV2, while DENV1 and DENV3 are more closely related [29]. The DENV genome of each of the serotypes comprises ≈ 11 kb of positive-sense, single-stranded RNA containing three genes that encode for 3 structural proteins: envelope (E), membrane (M) and capsid (C), and seven genes that encode for 7 non-structural proteins: NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5. Recently, it has been reported that the dengue NS1 antigen might be useful in early and precise diagnosis of acute phase of dengue illness, and the dengue NS1 antigen as well as the dengue specific IgM ELISA might be helpful in the diagnosis of dengue [30].

The DENV causes various clinical symptoms: from asymptomatic or undifferentiated fever, called DF, to fever with plasma leakage, called, DHF, and some of the cases of DHF expresses even more severity, known as DSS, leading to death [31,32]. As described by Guzman., *et al.* [3], the key clinical terms related to DENV infections include: DF: an imprecise feverish sickness characterized with fever and the presence of other symptoms, such as headache, rash, retro-orbital or ocular pain and myalgia; DHF: described with elevated permeability of blood vessels, leakage of plasma and bleeding, thrombocytopaenia and fever (as per 1997 WHO classification) [33]; dengue with warning signs: at the end of the febrile period, patients may experience signs or symptoms suggestive to loss of fluid, associated with capillary leakage and severe abdominal pain, with the requirement of prompt fluid replacement (as per the 2009 WHO classification) [34]; severe dengue: featured with shock or respiratory distress and severe plasma leakage, bleeding or severe organ involvement, such as myocarditis, encephalitis and severe hepatitis, with or without shock or bleeding (as per the 2009 WHO classification) [34].

Disease vector

In most of the South Asian parts, including India, two mosquito species of the genus *Aedes: Ae. aegypti* and *Ae. albopictus*, are considered as the main vectors for DENV transmission. The geographical distribution of the DENV serotypes is typically parallel to that of the principal vector of dengue: *Ae. aegypti* [35]. The mosquito showed high affinity to human blood, high adaptation to urban human population and high vectorial capacity for all four dengue serotypes (DENV1 to DENV4), and due to its restricted flight range the female *Ae. aegypti* endures in a domestic setting, which contribute to the dissemination of dengue through 'human-mosquito-human' contact within communities [36].

Dengue epidemiology

The dengue epidemic activity radically hastened during 1970s and 1980s, lead¬ing to a global geographical extension of DENVs and mosquito vectors: *Ae. aegypt*i and *Ae. albopictus,* and thus, transmission of dengue viruses in the tropics and subtropics [3]. The global estimates of dengue burden relies on 3.6 billion people (i.e. over half of the world's population) living in high risk areas of DENV infection, with about 390 million DENV infec¬tions, 96 million symptomatic cases, 2 million with severe illness and 21,000 annual deaths; the highest incidence of DENV infection has been reported from Asia, among children of 5 - 15 years of age [3]. It has been estimated that 96 million DENV infection occurred worldwide in the year 2010, of which mostly reported from Asia, creating 70% of the global burden, while Africa bore 16% of the global burden [37].

A record of Indian annual average dengue cases were 20,474 and 132 deaths from the infection, in 2006 - 2012; according to the NVB-DCP report, in India in 2015, the affected areas were Delhi, Punjab, Haryana, Gujrat, Karnataka and Kerala, with 4000 - 15,000 cases and 9 - 60 deaths [38,39]. It has been reported that India contributes to 34% (≈ 33 million infections) of the entire global dengue threat leading to endemic in urban areas [6]. The DENV was first isolated in Japan, in 1943, and in Kolkata in 1944 from serum samples of US soldiers [40], while the first evidence of the occurrence of DF was reported in 1956, from Vellore, India [41], and since then sporadic outbreaks of the disease have been reported from various states of the country, including West Bengal [40].

Diagnosis of infection

The use of good diagnostic tools for DENV infection, this is vital for laboratory confirmation of DHF/DSS, including the determination of number of case fatalities, the serotypes/strains involved, and to get information of total incidence from dengue epidemics. Dengue diagnosis is greatly important in research on host, virus and the vector characteristics, for determining the epidemiological conditions that influence the disease pathogenesis as well as vaccine development and evaluation [42,43]. The National Vector Borne Disease Control Program (NVBDCP), Government of India, recommended the application ELISA-based antigen detection test (NS1) in the diagnosis of cases from Day 1 onwards and antibody detection test (IgM), capture ELISA, in the diagnosis of cases after Day 5 of disease onset in order to confirm the DENV infection [42]. The dengue NS1 antigen detection has potential importance to screen the patient samples in early acute phase, while anti-dengue IgM antibody detection can help in diagnosing the severity of the disease due to the fact that IgM appear in the phase of DHF and/or DSS [44].

Treatment and management

The management of DF is symptomatic and supportive. In the absence of any effective antiviral drug for dengue, the prescription of bed rest and plentiful liquids and electrolytes, by oral route, can be crucial in determining the patient's outcomes, during the 24h, and analgesic and antipyretic drugs (viz., paracetamol; neither aspirin nor non-steroidal anti-inflammatory drugs should be taken, since it may cause gastritis, vomiting, acidosis, platelet dysfunction and severe bleeding complication), can be prescribed with usual dosage for children and

adults. Finally, the fluid therapy is best way to the DENV infection management and is applied based on disease severity [3]. The infected cases are needed to be observed under clinical supervision for 24 - 48h, in DHF endemic areas, until they become afebrile without the application of antipyretics, and after hematocrit values become stable with platelet count of ≥ 50,000/ml [45].

LDH isoforms and their role in DENV and GAS infection detection

The lactate dehydrogenase (LDH) is a intracellular cytoplasmic tetrameric enzyme which, along with the coenzyme, NAD+, catalyzes the inter-conver¬sion of lactate and pyruvate, and LDH has also seemed as an indicator of diseases and tissue injury [46]. The LDH enzymes are constituted with four polypeptide subunits, which may be of same kinds: heart (H) subunits, as in LDH1, and muscle (M) subunits, as in LDH5 (and thus these are homotetrameric isoforms), or different kinds: LDH2 (3H1M), LDH3 (2H2M) and LDH4 (1H3M), and are regarded as heterotetrameric isoforms [47]. The liver damage results in increased activity of the serum LDH5 isozyme, while kidney damage produced increased activity of LDH1 and LDH2 [48]. It is imperative to recognize that the half-life of serum LDH5 is 10h, and therefore, the increase in LDH5 may be temporary, and by contrast, the LDH1 has a half-life of almost 10-fold longer than that of LDH5, and thus, LDH1 increases may be detected for many days after the release of the enzyme into the intravascular compartment [49]. Among five LDH isoforms (LDH1 to LDH5), the LDH1 isoform, found in heart muscle, is the lightest one, while, the LDH5 is found in skeletal muscle and liver and is the heaviest of all the isoforms [47], while the LDH2 (3H1M), LDH3 (2H2M) and LDH4 (1H3M) show intermediate mobilities, and are found in varying degrees in many tissues, as has been reported by Sharma., *et al.* [47]. The LDH escaping of tissue damage results increased in its levels in serum.

The LDH isoforms have been regarded as the diagnostic marker of inflammatory disorders and of many non-communicable [50-52], as well as communicable diseases, such as the life-threatening bacterial infection [53,54]. The ARF has been the common diseases that tag on sore throat infection caused by GAS, whereby necrosis, degeneration, or inflammation of the respective damaged tissues result in increased level of serum LDH, because of the elevated rate of release of LDH from damaged tissues [47]. Sharma., *et al.* [47] demonstrated serum LDH isoforms through PAGE analysis in tuberculosis patients, signifying the effectiveness of LDH as a vital diagnostic marker of tuberculosis. They showed that the LDH1 isoform, which is the lightest one, originates in heart muscle, while the LDH2 and LDH3 isoforms, which show intermediary mobility, are found in varying degrees in different tissues [47]. It has been suggested that LDH, in association with clinical data, might be useful in preliminary screening of *Pneumocystis* pneumonia, in HIV infected patients, having 92.8% sensitivity and 83.9% specificity [55]. It has been reported that the elevated total serum LDH levels as well as LDH isozyme electrophoreses in serum samples of GAS infection cases, compared to the normal, are useful as a 'follow-up marker' of recovery of GAS infection [56].

Villar-Centeno., *et al.* [51] determined the frequency of biochemical alteration, using accepted normal upper limits for LDH (570 U/L); LDH (U/L) 562.3 (524.9 - 599.7) in DF and 711.6 (612.6 - 810.6) in DHF, supports the association between development of DHF and early alteration in the levels of the serum LDH. In majority of patients (92%) LDH levels were more than 600 U/L, as reported by Ravishankar *et al.* [57], while Liao., *et al.* [58] reported a little lower level of LDH, both in DF and DHF, 213.68 U/L and 448.17 U/L, respectively. The LDH was detected very high (2013 - 1708 U/L) in DF with haemolytic anaemia [59]. As per the report of Perveen., *et al.* [51], at the time of hospital stay, 61.3% cases had DF, while 39.7% DHF, and serum LDH levels were higher in cases with DHF (mean: 618.38U/L ± 219), as compared to the cases with DF (mean: 316.45U/L ± 104); therefore, the high serum LDH can be used to predict outcome in dengue patients. A recent report depicted increased levels of serum LDH associated with DF and severe DF and, thus, serum LDH might be useful 'as an early predictive marker of dengue illnesses for patients requiring prompt therapies [26].

Concluding Remarks

The DF, the spectrum of clinical manifestations of which develop rapidly into its severe forms: DHF and DSS, has been considered a major mosquito-borne health problem worldwide. Since, no specific treatment protocol has been justified for any form of dengue, an early detection via serum LDH profiling and tracing biomarkers like serum cytokines and chemokines as well as prompt right to use to proper medical care might help manage DNV infection thereby lowering case fatality [46,60]. Development of registered dengue vaccine, such as CYD-TDV (also called Dengvaxia: a live attenuated recombinant tetravalent) and 'promising advances in vector control technology interventions' might aid in global dengue prevention and management acts (WHO, 2016) [61].

On the other hand, GAS cause severe invasive infections: necrotizing fasciitis, meningitis and endocarditis, while the scarlet fever and STSS include the systemic response to bacterial toxins circulating in patients' body. An early diagnosis and treatment remain the vital factors in accomplishing successful control of devastating diseases from GAS infection, and therefore, global surveillance of this bacterial pathogen is strongly recommended for effective vaccine development.

Conflicts of Interest

The authors declare no conflict of interest.

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