LACTATE DEHYDROGENASE ISOENZYME PROFILES IN GROUP A STREPTOCOCCAL INFECTION

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INTRODUCTION

The group A streptococci (GAS), also known as Streptococcus pyogenes, are β-hemolytic gram-positive bacteria having the capacity to cause several life-threatening infections to humans, leading to 5.17x10^5 deaths per year globally, in addition to 2.33x10^5 deaths caused by rheumatic fever. The GAS though can be carried asymptomatically by human subjects, also can cause mild skin (impetigo: blistering skin infection) and mucosal (pharyngitis: strep throat) to severe life threatening infections: post-streptococcal glomerulonephritis, necrotizing fasciitis (NF), streptococcal toxic shock syndrome (STSS) and rheumatic fever (RF). Initiation of the apposite therapy might decrease the severity and clinical outcome of the disease, requiring proper detection and diagnosis of GAS infection in order to provide prompt treatment.

The microbiological diagnosis, through the isolation of β-hemolytic streptococci from blood cultures, as well as the serological diagnosis, through antibody detection (anti-streptolysin O and anti-DNase B), confirm the GAS infection, in the laboratory. The serum lactate dehydrogenase (LDH) is widely distributed in tissues such as liver, kidney myocardium, skeletal muscle and extracellular fluids, in human body. The RF 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 LDH in serum, due to elevated release rate of LDH from tissues.

The LDH has been studied as a diagnostic marker of non-infectious as well as infectious diseases, including bacterial infection. The infection with bacterial pathogens including group A streptococci causes a number of diseases, in humans, with multiple organs involvement and tissue damages leading to the secretion of LDH releasing into the blood. The serum LDH can thus be measured and could be considered as an indicator of the existence of illness due to pathogenic infection, including GAS. However, no earlier report has been made about the correlation between increased serum LDH levels and GAS infection, in terms of ASO positivity. The current study, therefore, evaluates the possible association between increased serum LDH level and GAS infection in comparison to the healthy controls, and to explore the serum LDH isoforms in GAS infection, for the first time, from this part of the globe.

MATERIALS AND METHODS

Serum samples

A retrospective collection of serum samples (n = 11) were made from blood of cases (adults: 22 – 55 years of age, both males and females) with GAS infection, in terms of anti-Streptolysin O (ASO) positivity, at Kishanganj Medical College, Kishanganj; serum samples (n = 9) from normal individuals were utilized as
the controls. The subjects were not directly involved in the study, and hence there was no need of application of consent from cases and controls, and no ethical clearance was needed because of the retrospective procurement of samples, for the current study.

Estimation of serum LDH

The serum samples, from cases (GAS infection) as well as the controls (normal individual) were subjected to total LDH estimation using standard protocol, in fully automated analyzing machine (Selectra Pro S, ELItech). The estimated LDH values were expressed in IU/L. The serum LDH levels of ≥320 IU/L were defined as elevated, since this is the upper limit of normal in the laboratory.

Profiling of LDH isoforms by PAGE

From each of the serum samples procured, an equal quantity of LDH was laden on to 7.5 % native PAGE to resolve the LDH isoforms in the cases (GAS infection) and the control (normal) serum samples. After electrophoresis (at 120 V for 4 h), the gel was developed for definite staining of LDH. Briefly, the gel was dipped in LDH staining dye, in a staining box, containing NAD (10 mg), NBT (10 mg), PMS (1 mg) and lithium lactate (5 ml) as the substrate, and kept in the dark, at room temperature, for ≈ 20 min, till LDH bands are visualized. The gel was then washed with distilled water, destained, photographed and stored in solution of glacial acetic acid (7%) and glycerol (10%) in distilled water (83%).

Statistical analysis

The study results, in terms of the total serum LDH levels, between GAS infection cases and healthy controls were compared following t-test, while between male cases and female cases as well male controls and female controls by F-test. The p values ≤ 0.05 were considered significant.

Table 1: Total serum LDH (lactate dehydrogenase) values in GAS (group A Streptococcus) infection cases and in healthy controls

<table>
<thead>
<tr>
<th>LDH values (mean ± SD; IU/L)</th>
<th>GAS infection cases</th>
<th>Healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>729.86 ± 161.46*</td>
<td>455.75 ± 74.60**</td>
<td>152.80 ± 32.46'</td>
</tr>
</tbody>
</table>

*p value: <0.0001; ** p value: <0.01; *p value: 0.67; † p value: 0.23
SD: standard deviation; * and † express p values by t-test; * and †† express p values by F-test

Figure 1: The serum LDH (lactate dehydrogenase) isoforms in GAS (group A Streptococcus) infection cases and healthy controls. Lane 1-3, 5 & 6: GAS infection cases; lane: 4 & 7: healthy controls. Note the staining intensity of LDH bands (isoforms) in the gel.

RESULTS AND DISCUSSION

The GAS, with broad array of human illnesses (superficial to invasive infections), causes rapid disease progression through their abundance in the lungs, extensive and severe pulmonary necrosis, and virulence factor (M proteins, toxins, proteases and DNases) profiles of the streptococci. Such virulence factors that include streptokinase (~50 kDa), cysteine protease (28.5 kDa) and superantigenic toxins (24–28 kDa), on penetration in to the body, cause invasive illnesses: NF and STSS. GAS also causes RF (throat culture positive for S. pyogenes, anti-streptolysin-O positive, and presence of carditis), and glomerulonephritis, which have been considered as serious problems in developing countries. An early therapeutic institution with intravenous immunoglobulin are considered in cases invasive infection including NF and STSS, requiring prompt and proper detection of GAS infection. The diseases with multiple organ involvement result tissue injuries inducing the cells to release excessive LDH, which transported into the blood stream, thereby causing elevated serum LDH, compared to the normal levels, and thus it has been hypothesized that the total serum LDH or the individual LDH isoforms may be utilized as the prognostic and/or diagnostic indicator of GAS infection.
In the current study, the total serum LDH isofoms have been estimated in GAS infection cases, confirmed in terms of ASO positivity, and the corresponding healthy controls (individuals whose serum showed negativity to ASO test) to justify the helpfulness of serum LDH as an investigative marker for GAS infection cases with RF. The total serum LDH levels in GAS infection cases were > 320 IU/L, both for males (mean: 729.86 ± 161.46 IU/L; range: 482 – 862 IU/L), and females (mean: 455.75 ± 74.60 IU/L; range: 370 – 540 IU/L), while among the normal groups the LDH values ranged from 152.80 ± 32.46 IU/L, for males, to 160.75 ± 40.33 IU/L, for females (Table 1); therefore, there was an significant elevation of total serum LDH levels in cases compared to the LDH levels in control groups (p value: < 0.01). The elevated LDH level was associated with bacteria cultures from synovial fluid having positive for the growth of Pseudomonas aeruginosa, Staphylococcus aureus and Enterococccus sp. as the sepsis causing agents11. Sameera et al.21 reported elevated serum LDH levels in typhoid cases indicating the diagnostic importance of total serum LDH and the usefulness such parameter (evaluation of serum LDH levels) in the determination of clinical and prognostic features of the illness.

The elevated serum LDH levels have been reported to be the diagnostic markers of various illnesses in humans, including the life-threatening bacterial infection10. The report on LDH isofoms analysis has, however, been scanty6. The serum LDH profile of cases and controls (in terms of GAS infection) has been determined in order to corroborate the intensity of LDH bands in the stained gel (Figure 1), with the estimated LDH levels in both kinds of test serum samples (cases and controls). The all serum samples from ASO positive cases of GAS infection had elevated level of LDH1, LDH2 and LDH3 isofoms, on the basis of staining intensity, when compared to these LDH bands in normal serum samples. The phenomenon of increased levels of LDH1, LDH2 and LDH3 isofoms (lane: 1, 2, 3, 6, and 7, loaded with serum samples from GAS infection cases) has evidently been proved, in the current study (Figure 1). Sharma et al.9 demonstrated serum LDH isofoms through PAGE analysis in tuberculosis patients, signifying the effectiveness of LDH as a vital diagnostic marker of tuberculosis.

It has been reported that the LDH measurement might be a valuable tool in identifying human illnesses with excessive tissue damage20. The increase in serum LDH levels in typhoid cases, as has been reported by Sameera et al.21, might be due to the necrosis in lymphoid tissues of a typhoid fever patients compared to the healthy controls. The LDH1 isofom, which is the lightest one, originates in heart muscle, while the LDH2 and LDH3 isofoms, which show intermediary mobility, are found in varying degrees in different tissues4. In this study, the ASO positivity, in GAS infection cases, caused an increased expression of three LDH isoenzymes (LDH1, LDH2 and LDH3 isofoms), and thus plausibly that these LDH isofoms, from GAS induced corresponding tissue injuries, might have been disseminated into blood elevating the serum LDH isofoms.

CONCLUSION

The current study explores the elevated total serum LDH levels as well as LDH isozyme electrophoretotypes in serum samples of GAS infection cases and in corresponding healthy controls, indicating the diagnostic importance of total serum LDH and the LDH isofom patterns in clinical practice for GAS infection, and the usefulness of serum LDH as a ‘follow-up marker’ of recovery of GAS infection among patients having chemotherapeutic treatment too.

REFERENCES


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