Serum Lysozyme (Muramidase) Levels in Intra-Abdominal Abscesses: An Experimental Study

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Abstract The role of serum lysozyme in the presence of intra-abdominal sepsis was studied to act as an adjunct to various other modalities used for diagnosing intra-abdominal abscesses. Lysozyme (muramidase) is a bacteriolytic enzyme located within phagocytic cells including leukocytes. We measured serum concentrations of lysozyme by a standard turbidimetric method on three groups of murine models. One group underwent caecal ligation and puncture (CLP), the second group underwent a sham operation, and the third group was used as controls. In mice with intra-abdominal abscesses secondary to caecal ligation and perforation ($n=30$), the serum lysozyme levels were increased compared to the sham-operated mice ($n=30$) and controls ($n=30$) ($p<0.001$). In this study, serum lysozyme levels have a high sensitivity and specificity related to the presence of an intra-abdominal abscess in mice. Clinical studies are required to demonstrate its role in humans.

Keywords Lysozyme · Muramidase · Intra-abdominal abscesses

Introduction

Intra-abdominal abscesses are associated with a high morbidity and mortality [1]. A surgical dilemma occurs when the diagnosis is elusive and unconfirmed by various imaging modalities [2, 3]. There is a need for an effective, accurate, and low-cost mechanism for detecting intra-abdominal abscesses. Lysozyme (muramidase), a bacteriolytic enzyme found in serum as well as other body fluids and tissues, has been found to be an indicator of macrophage mediated host response in a few studies and correlates well with white cell death and a high turnover rate [4, 5]. The term “lysozyme” is derived from the ability of this enzyme to dissolve the walls of certain bacteria. It has been designated muramidase, since it is known to facilitate the hydrolysis of a β -1-4-glycosidic bond between *N*-acetylglucosamine and *N*-acetylmuramic acid in bacterial cell walls.

Increase in serum lysozyme has been previously reported in certain chronic inflammatory conditions, inflammatory bowel diseases, hematological disorders, and renal disorders [6–8]. The role of serum lysozyme for detecting intra-abdominal abscesses in a clinical trial has not yet been reported. Results from an experimental study may demonstrate a need for a clinical study on the relation of serum lysozyme and intra-abdominal abscesses.

Materials and Methods

The study was approved by the Institutional Animal Ethics Committee. *Wistar albino* mice were reared in our laboratory conforming to the specifications laid out by the Indian Council of Medical Research (ICMR).

Ninety healthy mice were randomly divided into three equal groups. Thirty mice underwent a midline laparotomy

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and caecal ligation and puncture (CLP) [9] with 3-0 silk and the incision thereafter closed with 4-0 nylon. Thirty underwent a sham operation and 30 were used as controls. The serum lysozyme activity was measured after 7 days by venepuncture using the common iliac vein following the confirmation of the presence or absence of an intra-abdominal abscess by re-exploration.

All mice in the CLP group ($n=30$) were found to have developed intra-abdominal abscesses on re-exploration. None of the mice in the sham-operated group ($n=30$) were found to have developed intra-abdominal abscesses on re-exploration.

The lysozyme activity was measured with a standard turbidimetric technique by the use of light spectrophotometry [10]. Statistical analysis was done using ANOVA tests.

Results

The three groups of mice were well matched with respect to weight. The mean lysozyme activity was 26.65 ± 1.13 in the experimental group, 12.19 ± 0.61 in the sham group, and 13.89 ± 0.60 in the control group (Fig. 1). There was a statistically significant rise in the serum lysozyme activity in the experimental models (all of which had intra-abdominal abscesses) as compared to the sham and control models ($p < 0.001$). At cut-off levels of the lysozyme activity, 15 units/liter, the sensitivity was 100 %, specificity 68.3 %, positive predictive value 61.2 %, negative predictive value 100 %, and accuracy 78.9 %. At cut-off levels of 20 units/liter, the sensitivity was 83.3 %, specificity 100 %, positive predictive value 100 %, negative predictive value 92.3 %, and accuracy 94.4 % (Fig. 2).

Discussion

Lysozyme is an enzyme that was first identified and named by Alexander Fleming, who recognized its bacteriolytic properties [11]. It is abundant in a number of secretions, such as tears, saliva, and mucus, and also present in cytoplasmic granules of

Fig. 1 Increased lysozyme activity (Units/L) in 'experiment' group

Performance of Lysozyme as a Diagnostic Test

Fig. 2 Performance of lysozyme as a diagnostic test

the polymorphonuclear neutrophils. Lysozyme can act to some extent as an innate opsonin, or as an actively lytic enzyme. As an innate opsonin, it functions by binding to the bacterial surface, reducing the negative charge and facilitating phagocytosis of the bacterium before opsonins from the acquired immune system arrive at the scene, thus making it easier for phagocytic white blood cells to engulf bacteria. Lysozyme in the plasma comes chiefly from the degradation of granulocytes and monocytes and its concentration reflects the turnover of these cells. Increases are seen in benign diseases like inflammatory bowel diseases, many blood disorders like polycythemia vera, multiple myeloma, and malignant processes like leukemias [6–8, 12–14]. Various studies have been performed on the estimation of serum lysozyme for tuberculous pleural effusion, sarcoidosis, and other chronic inflammatory conditions [6–8].

In this study, the mouse model was chosen because of its close genetic and physiological similarities to humans. In addition, they are small, easy to maintain in a laboratory, and have a short breeding cycle. Only one experimental study has been conducted relating serum lysozyme to intra-abdominal abscesses [15]. This study included 20 mice in a CLP group and 20 mice in a sham-operated group. The results showed a statistically significant elevation in the serum lysozyme levels in the mice with intra-abdominal abscesses.

In our study, the diagnostic significance of the serum lysozyme activity was evaluated at different ranges. When the serum lysozyme activity was >20 units/liter, lysozyme proved to be an excellent diagnostic marker for intra-abdominal abscesses. In our study, serum lysozyme levels have a high specificity and sensitivity related to the presence of an intra-abdominal abscess and is an indirect measure of the sequestration of leukocytes to the site of an established or developing collection of intra-abdominal pus.

Therefore, where the diagnosis of intra-abdominal abscess remains unconfirmed by radiological and routine laboratory tests, the increase in serum lysozyme activity may thus be a useful adjuvant to determining the presence of an intra-abdominal abscess [15]. We know that lysozyme levels go up in the presence of infection [8]. Quantitative rise of lysozyme in other infection models compared to our results will be another way to substantiate our results as well as categorize the sensitivity of this test for the presence of localized pus as compared to generalized sepsis. Even if the levels of lysozyme are raised in all infective conditions, the clinical scenario of patients with generalized sepsis and occult abscesses is very different. Thus a raised lysozyme associated with the clinical suspicion may be a good indicator for the diagnosis of intra-abdominal abscess. How well the level of serum lysozyme correlates with the amount of pus will also need further studies.

Conclusion

The estimation of serum lysozyme activity seems to be, potentially, a very useful diagnostic marker to identify critically ill patients with an occult intra-abdominal abscess. Although promising, this test requires further evaluation before its routine use can be recommended.

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