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The Evaluation of Correlation Between Serum High Mobility Group Box 1 (HMGB1) with Severity of Acute Cholecystitis

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Abstract: The aim of this case control study was therefore to evaluate the correlation of preoperative serum High Mobility Group Box Protein-1 (HMGB1) levels in patients with various types of acute cholecystitis. This study was carried out from April, 2011 through May, 2012 and included 35 healthy control group and 68 patients (45 as mild and 23 as sever form) who presented at the Emergency Department of Valiasr Training and Research Hospital in Iran with acute abdominal pain who were clinically and sonographically diagnosed as acute cholecystitis. Clinical and demographical information as well as sonographic and operative findings were assessed in all patients. HMGB1, Liver Function Test (LFT), Complete Blood Count (CBC), amylase and lipase blood samples were determined on admission and 48-72 h after cholecystectomy. Patients with diagnosed acute cholecystitis were categorized into 2 groups of severity of infection based on operative and pathologic findings: mild cholecystitis (n = 45) and severe cholecystitis (n = 23). Although, the median level of HMGB1 was significantly higher in patients with acute cholecystitis compared to control group ($p<0.007$). There was not statistically significant difference in HMGB1 levels between patients with different types of acute cholecystitis. HMGB1 can be used as a diagnostic tool in acute cholecystitis but was not clear correlation with severity of acute cholecystitis.

Key words: Cholecystitis, HMGB1, severity, LFT, CBC, Arak

INTROUDUCTION

Acute cholecystitis is secondary to gallstones in 90-95% of cases (Brunicardi *et al*., 2010). Autopsy reports have shown a prevalence of gallston from 11-36% (Brett and Barker, 1976). Acute acalculous cholecystitis is a condition that typically occurs in patients with other acute systemic diseases. In the most severe cases, this process can lead to ischemia and necrosis of the gallbladder wall (5-10%) (Brunicardi *et al*., 2010). Acute gangrenous cholecystitis results in formation of an abscess or empyema Within the gallbladder. Right upper quadrant pain is the most common symptom of acute cholecystitis. Ultrasound is the most useful radiographic test for diagnosing acute cholecystitis with sensitivity and specificity of 85 and 95%, respectively (Acosta and Adams, 2007).

HMGB1 is viewed as a proinflammatory cytokine due to its active secretion by innate immune cells such as neutrophils, monocytes and macrophages (Andersson *et al*., 2000; Park *et al*., 2003). Sims *et al*. (2010) have demonstrated that HMGB1 released actively following cytokine stimulation. Van Zoelen *et al*. (2007) has studied systemic and local level of HMGB1 in severe infection. Kocsis *et al*. (2009) have demonstrated circulating level was significantly higher in patients with severe acute pancreatitis. Despite the multiple modern diagnostic tools current available, diagnosis of sever forms of acute cholecystitis (gangrenous) maybe not clearly apparent especially in patient who admitted in ICU wards and because of decrease level of consciousness physical examination maybe unreliable.

However, recent studies have revealed that some inflammatory markers such as the HMGB1 may be detectable already in the early state of infection (Kocsis *et al*., 2009). Because lacking studies on HMGB1 in acute cholecystitis has been made. The main aim of this study was to compare the Levels of HMGB1 between patients with acute cholecystitis of different severity levels.

Furthermore, a secondary aim was to examine the ability of HMGB1 as marker for early detection of cholecystitis.

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MATERIALS AND METHODS

Study populations: The protocol was approved by the Research Ethics Committee of Arak School of medicine. This study was carried out from April, 2011 through May, 2012 and included 68 patients who presented at the emergency department of Valiasr Training and Research Hospital in Iran with right upper abdominal pain who were clinically and by sonography diagnosed as acute cholecystitis and based on operative and pathological findings included in mild and severe types (mild included simple inflammation of gallbladder and severe forms included gangrenous, empyema, emphasematous and perforated gallbladder). Full blood counts and biochemistry, LFT and HMGB1 were performed on patients who had suspicious of acute cholecystitis and a second blood sample obtained 48-72 h after cholecystectomy.

A healthy control group consisted of 35 healthy persons who had no complaints and who did not conform to the exclusion criteria and blood sample HMGB1 were obtained.

Inclusion and exclusion criteria: All of the patients underwent operations for acute cholecystitis on the basis of the history, physical findings and sonography. Postoperatively, the removed gallbladder was sent for histopathological examination. Cases where the histopathology was not consistent with acute cholecystitis excluded from the study. The exclusion criteria for entry into the study were haematological disorders, other acute or chronic infections, pancreatitis, cancer, prior antibiotic therapy, an age <18 years, pregnancy, hepatic diseases, immunodeficiency and other known inflammatory conditions. None of the patients had received prior anticoagulant medications, nonsteroidal anti-inflammatory corticosteroids or immunosuppressant medication drugs or oral contraceptives.

Laboratory assays: Preoperative CBC and biochemistry, LFT were obtained from the patients. Pre and 48-72 h postoperative HMGB1 plasma were collected into microtube after centrifuged at 1300 g for 10 min and stored at -80°C until the analysis. HMGB1 levels were measured with a commercially available enzyme inked immunosorbent assay (HMGB1ELISA kit; Shino-Test Corporation, Tokyo, Japan). The measuring range was 1-80 ng mL^{-1}, the coefficient of variation being <10%. Recovery of HMGB1 in this ELISA was 80-120%. HMGB1 was analyzed in the Laboratory of Clinical Immunology, Arak School of Medicine.

Statistical analyses: A total of 68 patients and 35 healthy persons were included in this study and were separated into 3 groups. The 1st group (Group 1) consisted of healthy persons (n = 35), the 2nd group (Group 2) consisted of mild acute cholecystitis patients (n = 45) and the 3rd group (Group 3) consisted of severe patients (n = 23). The Statistical Package for Social Sciences (SPSS) 16.0 for windows was used to analyze the data in terms of mean±Standard Deviation (SD). Based on Kolmogrovo-Smirnov test distribution of HMGB1with SD of 5% was not normal in three groups, therefore to compared median values HMGB1 in 3 groups, non parametric Kruscal-Wallis test was used . That difference in median values of HMGB1 was statistically meaningful in 3 groups comparison ($p<0.007$)(Fig. 1).

Median values of HMGB1 in the control group was 2/683±1/181 ng mL^{-1} that more less than value in mild

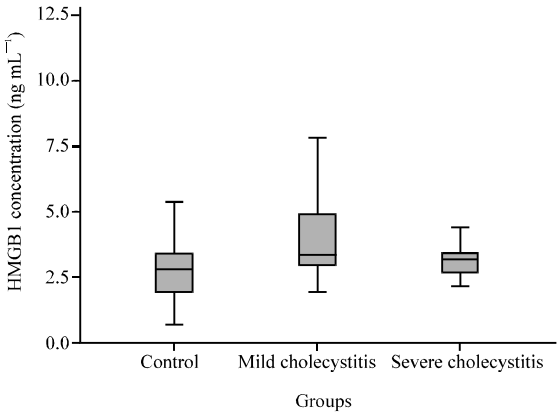

Fig. 1: The HMGB1 level of the patients and controls groups before cholecystectomy

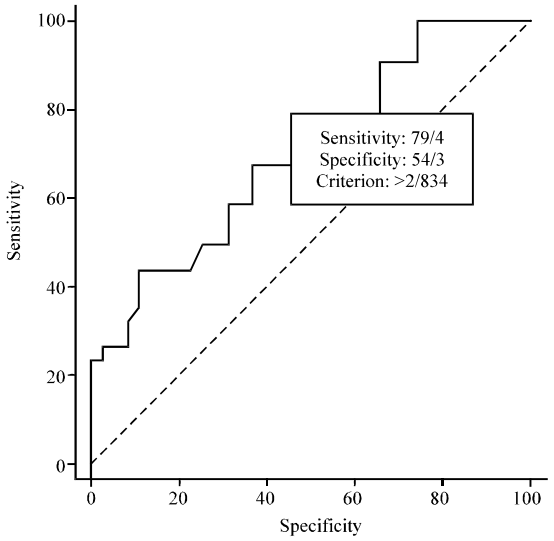

Fig. 2: Receiver Operating Characteristic (ROC) curve of High Mobility Group Box protein 1 (HMGB1)

and severe groups. Pre and post operative HMGB1 level compared with t-paired test that in both groups of mild and severe lowered after cholecystectomy ($p<0.001$). But, HMGB1 level compared in mild and severe groups that no statistically meaningful difference observed ($p>0.05$). Receiver Operating Characteristic (ROC) curve analysis was used to identify optimal cut-off values of HMGB1 between control and disease groups (mild and severe). Sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were calculated according to standard methods. The $p<0.05$ were considered statistically significant (Fig. 2).

RESULTS AND DISCUSSION

Patient characteristics: Of the 68 patients who underwent cholecystectomies, 20 were male and 48 were female and of the healthy group, 14 were male and 21 female. Demographic and clinical characteristics with regard to age, gender distribution, WBC and HMGB1 levels are shown in Table 1.

The average HMGB1 value of the healthy group was 2/683±1/181 ng mL^{-1}. When the groups were compared, the HMGB1 serum levels were significantly lower ($p<0.001$) in the healthy control group. When all 68 patients with acute cholecystitis were compared by a t-test with the healthy group, the higher values of HMGB1 in the acute cholecystitis patients were statistically significant ($p<0.0007$). But no statistical difference was found between group 2 and 3 ($p>0.05$).

The diagnostic value of HMGB1 levels was investigated by calculating ROC curves. For the diagnosis of acute cholecystitis, the best cut-off point was at >2/834 ng mL^{-1}. The calculated sensitivity, specificity, positive predictive value and negative predictive value were calculated a 79/4, 54/3%, 1/74 and 0/38%, respectively (area under curve = 0.71) (Fig. 2).

In this study, researchers investigated potential variations in the blood levels of HMGB1 levels that might occur in patients with acute cholecystitis when compared with healthy persons. The overall aim was to determine whether this blood component might have a place in the diagnosis of acute cholecystitis and correlation with severity of disease. The second aim of the study was to facilitate the diagnosis of acute cholecystitis as the recognition of that maybe difficult in Intensive Care Unit (ICU) patient.

To the knowledge, no studies reported in the literature have yet evaluated an association between HMGB1 and acute cholecystitis.

High Mobility Group Box 1 protein (HMGB1), originally characterized as a nuclear DNA-binding protein and has also been described to have an extracellular role when it is involved in cellular activation and proinflammatory responses (Sha *et al*., 2008). High Mobility Group Box 1 (HMGB1) is a highly conserved, ubiquitous protein present in the nuclei and cytoplasm of nearly all cell types.

Table 1: Clinical characteristics of acute cholecystitis patients and controls

Clinical characteristics	Group 1 (n = 35)	Group 2 (n = 45)	Group 3 (n = 23)
Sex (M/F)	14/21	10/35	10/13
Age (year) (median, range)	52/9 (±19/46)	56/8 (±18/96)	59/74 (±19/26)
WBC ($\times 10^9$ L^{-1})	-	8/871(±2/86)	11/5 (±2/446)
Amylase	-	46/72±19/7	42/41±17/20
Lipase	-	40/37±71/3	28/34±12/13
HMGB1 (ng mL^{-1})			
Pre-op.	2/683 (±1/181)	4/414 (±2/688)	3/483 (±1/69)
Post-op.	-	3/683 (±1/37)	2/688 (±578)

Group 1: Control group, n = 35; Group 2: Acute mild cholecystitis patients; Group 3: Acute severe; cholecystitis patients; M: Male; F: Female; WBC: White Blood Cell count; HMGB1: High Mobility Group Box protein 1

They recently discovered that HMGB1 is secreted into the extracellular milieu and acts as a proinflammatory cytokine (Yang *et al*., 2005). High Mobility Group Box 1 (HMGB1), a nonhistone chromatin-associated protein is implicated as a mediator of both infectious and non-infectious inflammatory conditions (Fukami *et al*., 2009). It was discovered >30 years ago as a nuclear DNA-binding protein and was initially named for its characteristic rapid electrophoretic mobility in polyacrylamide gels (Johns, 1992).

Elevated levels of HMGB1 in serum and tissues occur during sterile tissue injury and during infection and targeting HMGB1 with antibodies or specific antagonists is protective in established preclinical inflammatory disease models including lethal endotoxemia or sepsis, collagen-induced arthritis and ischemia-reperfusion induced tissue injury (Yang *et al*., 2005). HMGB1 has been suggested to serve as a proinflammatory cytokine (Andersson and Tracey, 2003) and it has many organ-specific biological functions including induction of fever, anorexia and weight loss as well as cytokine production in the brain, acute lung injury and production of proinflammatory cytokines/mediators in the lungs, promotion of translocation in the gut, induction of arthritis and joint inflammation, modulation of heart rhythm and bactericidal effects (Yang *et al*., 2005). In severe sepsis, the kinetics of HMGB1 release may differ depending on the primary source of infection. In patients with severe infection, HMGB1 release predominantly occur at the site of infection (Van Zoelen *et al*., 2007). The circulating HMGB1 level was significantly higher in patients with severe acute pancreatitis (13.33±2.11 ng mL^{-1}) than in healthy controls

(0.161±0.03 ng mL^{-1}) or than in patients with mild pancreatitis (2.64±0.185 ng mL^{-1}) (Kocsis *et al*., 2009). There is a similar mechanism which is the increase of serum HMGB1 levels in patients with acute cholecystitis. It may be produced and released by macrophages/monocytes in response to inflammatory mediators. In acute cholecystitis, it is conceivable that the release of humoral mediators from the excessive activated macrophages/monocytes may lead to remote organ injury. As the released HMGB1 can cause the development of inflammation (Yang *et al*., 2001). Release of HMGB1 from activated macrophages/monocytes may participate in tissue inflammation in acute cholecystitis. For these reasons, serum levels of HMGB1 rise during acute cholecystitis. In this study, researchers have also studied correlation between an increase in HMGB1 levels and acute cholecystitis. This study has also shown that in acute cholecystitis, an increase in HMGB1 levels may occur with increase in WBC numbers. These findings have shown that HMGB1 serum levels may be used in the diagnosis of acute cholecystitis as a non-invasive indicator. But levels are not clear correlation with severity of disease.

CONCLUSION

In the results, although many auxiliary diagnostic tools are available for diagnosis of acute cholecystitis but none of them diagnosed severity of disease exclusively. Therefore, a need exists for new diagnostic tools that can support a diagnosis of severity of cholecystitis. Although, the study have not determined clear correlation HMGB1 serum levels with severity of disease but suggested larger studies with more cases.

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