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SERUM LEVELS OF C-REACTIVE PROTEIN, HEPCIDIN AND INTERLEUKIN-6 IN ACUTE CHOLECYSTITIS AND ITS CORRELATION WITH SEVERITY OF ATTACK AND RECOVERY TIME

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ABSTRACT **Background**. Prognostic markers for acute cholecystitis are not yet established in literature. **Aim**. To document levels of C-reactive protein, Hepcidin and Interleukin-6 in acute cholecystitis, and their correlation with disease severity and recovery time. **Methods.** This prospective observational study was conducted in a tertiary care hospital in north India. 41 patients with acute cholecystitis were included. Blood samples were collected for evaluation of the three markers at the time of admission and levels were correlated with duration of complaints, severity of acute cholecystitis, and the time taken for recovery. **Observations.** Levels are increased in patients with acute cholecystitis, and correlate with the total leukocyte counts at the time of admission, duration of pain before admission, disease severity, and time taken for recovery. **Conclusion.** The marker levels will not aid in diagnoses of acute cholecystitis, assess severity, help predict the disease course and time required for recovery.

KEYWORDS : Acute phase reactants; Biliary emergency; Biliary infection.

INTRODUCTION

Acute cholecystitis is one of the main biliary tract emergencies.(1) Its prevalence in North India is the highest amongst the general population in India. Early diagnosis and management of acute cholecystitis allows prompt treatment and reduces both morbidity and mortality.(2)

While the role of C-reactive protein has been studied in the diagnosis and severity of acute cholecystitis,(2-8) there have been no studies to correlate the levels of Hepcidin and Interleukin-6 with the severity of acute cholecystitis and the time taken for recovery.

The aim of our study was to document the levels of C-reactive protein, Hepcidin and Interleukin-6 in patients with acute cholecystitis, and to study the correlation between these levels and the severity of the attack, and the time taken for recovery from the attack of acute cholecystitis.

MATERIALS AND METHODS

This prospective observational study was conducted in the Department of Surgery in collaboration with the Department of Pathology in a tertiary care hospital in New Delhi, India. 41 consecutive patients meeting the Tokyo guidelines(2)(Table 1) for the diagnosis of acute cholecystitis attending the Accident and Emergency department of the hospital were included in the study.

Excluded from the study were patients with pancreatitis, choledocholithiasis, malignancy, patients with acute hepatitis, other acute abdominal diseases and chronic cholecystitis. Also excluded were patients requiring emergency surgery, and those patients with significant comorbid cardiac conditions, pregnancy, or using birth control pills or steroids.

A. Local signs of inflammation
(1) Murphy's sign, (2) RUQ mass/pain/tenderness
B. Systemic signs of inflammation
(1) Fever, (2) elevated CRP, (3) elevated WBC count
C. Imaging findings: imaging findings characteristic of acute cholecystitis
Suspected diagnosis: One item in A + one item in B
Definite diagnosis: One item in A + one item in B + C
Note: acute hepatitis, other acute abdominal diseases, and chronic cholecystitis should be excluded
RUQ right upper quadrant, CRP C-reactive protein, WBC white blood cell

Table 1. Tokyo guidelines.[2] Diagnostic criteria for acute cholecystitis.

The study was approved by the Institutional Ethics Committee and informed consent was taken from all patients.

All the patients in the study, diagnosed as acute cholecystitis and graded for severity according to the Tokyo guidelines(2)(Table 1 and 2), underwent a complete physical examination, complete haemogram and biochemical tests (including renal function tests, liver function tests, prothrombin time and serum amylase), ultrasonography of the abdomen, chest x-ray, and electrocardiogram.

Blood samples were collected from all patients for evaluation of C-reactive protein, Hepcidin and Interleukin-6 at the time of admission before the start of treatment.

A. 'Mild (Grade I)' acute cholecystitis does not meet the criteria of 'severe (Grade III)' or 'moderate (Grade II)' acute cholecystitis. Grade I can also be defined as acute cholecystitis in a healthy patient with no organ dysfunction and only mild inflammatory changes in the gallbladder, making cholecystectomy a safe and low-risk procedure.
B. 'Moderate (Grade II)' acute cholecystitis is accompanied by any one of the following conditions:
1. Elevated WBC count (>18000/mm2)
2. Palpable tender mass in the right upper abdominal quadrant.
3. Duration of complaints >72hr.
4. Marked local inflammation (gangrenous cholecystitis,

pericholecystic abscess, hepatic abscess, biliary peritonitis, emphysematous cholecystitis)
C. 'Severe (Grade III)' acute cholecystitis is accompanied by dysfunctions in any one of the following organs / systems
1. Cardiovascular dysfunction (hypotension requiring treatment with dopamine >5µg/kg per min, or any dose of dobutamine)
2. Neurological dysfunction (decreased level of consciousness)
3. Respiratory dysfunction (PaO2/FiO2 ratio <300)
4. Renal dysfunction (oliguria, creatinine>2.0 mg/dl)
5. Hepatic dysfunction (PT-INR >1.5)
6. Hematological dysfunction (platelet count <100000/mm3)

Table 2. Tokyo guidelines.[2] Criteria for mild, moderate, and severe acute cholecystitis.

The acute condition was treated by intravenous fluids, analgesics and antibiotics, and the patients were closely monitored. The patients' blood parameters were re-evaluated every 12 hours and before discharge. All the patients responded to the aggressive resuscitation and conservative management and urgent biliary drainage was not required in any of the cases.

Patients were discharged when there was complete relief of pain, fever and tenderness, along with normalization of the total leukocyte counts and acceptance of oral diet, and planned for elective laparoscopic cholecystectomy after six weeks.

Estimation of C-reactive protein, Hepcidin and Interleukin 6 were done in the following way:

C-reactive protein measurement .C-reactive protein (CRP) measurement was done using Calbiotech C-Reactive protein ELISA. The expected normal value of CRP according to this test kit was <10mg/L in both males and females.

Hepcidin measurement. Hepcidin measurement was done using hepcidin-25 (bioactive) ELISA of DRG instruments GmbH, Germany. The readings were made using automated 680 XR microplatereader at the Department of Pathology. The expected normal value for this kit was 1.0 to 39.3 ng / mL in both males and females.

Interleukin–6 measurement. Interleukin–6 measurement was done using the Diaclone IL-6 ELISA kits of Diaclone SAS, France. The expected normal value in this kit was <2pg/mL.

The levels of these markers was correlated with the patients clinical and laboratory data including the duration of symptoms, severity of the acute cholecystitis, and time taken for complete recovery.

The data was compiled and analysed using correlation and regression analysis statistical method.

OBSERVATIONS AND RESULTS

A total of 41 patients fulfilling the inclusion criteria were included in the study. The age of the patients ranged from 23 years to 70 years with a mean age of 39.63 years. The disease was seen to be more common in females: 31 out of 41 patients (75.6%) being females. The duration of complaints of the patients ranged from 1 to 4 days with a mean of 2.34 days. Out of these 41 patients of acute cholecystitis, there were a total of 9 (22%) mild cases, 18 (43.9%) moderate cases, and 14 (34.1%) severe cases when classified according to the Tokyo guidelines (Table 2).(2) The number of days of hospital admission in these patients ranged from 2 to 5 days, with a mean of 3.4 days.

The levels of C-reactive protein, Hepcidin and Interleukin-6 in the patients at admission are shown in Table 3. The correlation between these markers and the severity of acute cholecystitis, duration of complaints and the duration of admission is as shown in Tables 4-6.

As can be seen from the Tables 4-6, the C - reactive protein, Hepcidin and Interleukin-6 levels correlate significantly and directly with the duration of complaints, the severity of the disease, and with the number of days taken to recover from the attack.

Thus, from our study, it is seen that the levels of C-reactive protein, Hepcidin and Interleukin-6 are increased in the patients with acute cholecystitis, and their levels correlate with the levels of total leukocyte counts at the time of admission (significance level {2-tailed} <0.01 for all the three markers), duration of pain of acute cholecystitis before admission (significance level {2-tailed} <0.05 for all the three markers).

	Mean + SD	Range
C-reactive protein {mg/l}	82.776+ 107.239	3 – 322.5
Hepcidin {ng/ml}	65.261 + 48.748	7.2 - >140
Interleukin-6 {pg/ml}	22.223 + 26.304	0.21 – 77.5

Table 3.Levels of C-reactive protein, Hepcidin and Interleukin-6 in the patients at time of admission.

Grade	Mild	Moderate	Severe	P value	Mild vs Moderate	Mild vs Severe	Moderate vs Severe
No. of cases	9	18	14				
CRP{Median}	4.6	8.65	217.95	<0.001	0.207	<0.001	<0.001
Interleukin-6 { Median }	2.2	5.35	46.6	<0.001	0.237	<0.001	<0.001
hepcidin {Median}	16.4	38.65	217.95	<0.001	0.080	<0.001	<0.001

Table 4. Correlation between the marker levels and severity of acute cholecystitis.

	Total duration of presenting complaints at admission (days)			
	1 day	2 days	3 days	4 days
CRP levels (mean + SD)	8.2 + 10.21	158.2 + 176.06	102.16 +101.91	155.7 + 141.00
Hepcidin (mean + SD)	23.0 + 13.98	66.98 + 50.34	74.67 + 48.91	101.74 + 52.48
Interleukin-6 (mean + SD)	2.29 + 2.23	41.35 + 29.80	25.89 + 31.05	46.15 + 24.58

Table 5.Correlation between the marker levels and duration of complaints at admission.

There is significant correlation between the levels of these markers and severity of the disease. The mean levels of C-reactive protein (mg/L) in mild / moderate / severe cases is 12.44 / 32.19 / 192.8, the mean levels of Interleukin-6 (pg/L) in mild / moderate / severe cases is 5.22 / 10.6 / 49.43 and the mean levels of Hepcidin(ng/L) in mild / moderate / severe cases is 27.52 / 43.06 / 108.06. This shows that as the severity increases, the levels of these markers also increase.

	Total time taken for recovery (days)			
	2 days	3 days	4 days	5 days
CRP levels (mean + SD)	3	30.05 + 55.47	39.11 + 65.05	237.25 + 68.98
Hepcidin (mean + SD)	10.52	36.61 + 29.01	50.09 + 29.87	127.84 + 27.65
Interleukin-6 (mean + SD)	0.43	12.71 + 22.62	16.41 + 19.580	57.16 + 15.43

Table 6.Correlation between the marker levels and duration of admission (time for recovery).

The elevated levels also correlate with the number of days of admission, meaning that the period of recovery is longer if these values are higher and vice versa.

DISCUSSION

The use of definite diagnostic guidelines help in prompt and appropriate management of patients with acute cholecystitis, thus showing the importance of having structured diagnostic guidelines. The Tokyo guidelines were formulated based on the best available evidence and the experts' consensus achieved at the International Consensus meeting for the Management of Acute Cholecystitis and

Cholangitis, held on April 1–2, 2006, in Tokyo, and revised in 2012.(2) According to the Tokyo guidelines, diagnosis is the starting point of the management of acute cholecystitis, and prompt and timely diagnosis should lead to early treatment and lower mortality and morbidity. C-reactive protein (CRP) is not commonly measured in many countries. However, because acute cholecystitis is usually associated with an elevation of CRP level by 3 mg/dl or more, CRP was included in the guidelines. Diagnosis of acute cholecystitis by elevation of CRP level (3 mg/dl or more), with ultrasonographic findings suggesting acute cholecystitis, has a sensitivity of 97%, specificity of 76%, and positive predictive value of 95%.These guidelines have cleared validation studies and therefore are most appropriate in diagnosing acute cholecystitis.(2)

There have been a number of studies that have attempted to correlate the severity of acute cholecystitis with a variety of parameters.(3-9) According to a study by Schafer, levels of C-reactive protein correlates with the severity of acute cholecystitis with levels > 10 mg/dl indicative of tissue necrosis.(3)

It has been observed in studies that patients with advanced acute cholecystitis were significantly older, were predominantly male, and had a prolonged duration of symptoms as well as increased C-reactive protein levels and WBC counts.(4)

In a prospective clinical study by Juvonen T et al on diagnostic accuracy of ultrasonography and C reactive protein concentration in acute cholecystitis,ultrasonography correctly classified 86 of 108 patients with acute cholecystitis(79%). They conclude that the combination of ultrasonography and measurement of C reactive protein concentration is recommended in the routine investigation of all patients with suspected acute cholecystitis, and recommended that serum C-reactive protein concentrations should be monitored regularly to select those patients who require emergency operation.(5)
In a prospective study by Nikfarjam M et al on the outcomes of contemporary management of gangrenous and non-gangrenous acute cholecystitis, he states that the risk factors associated with gangrenous cholecystitis(a more severe form of acute cholecystitis) included older age, diabetes, temperature of >38 °C, tachycardia, detection of muscle rigidity on examination and greater elevations in white cell count, C-reactive protein (CRP), bilirubin, urea and creatinine. Thus, C - reactive protein is an important marker of severe cholecystitis.(6)

In a prospective study by Teckchandani N, he found that male sex, preoperative duration of symptoms, WBC counts, serum alkaline phosphatase, serum amylase, and serum C-reactive protein were significant predictors of histopathological severity of acute cholecystitis. Male sex and serum C-reactive protein levels >3.6 mg/dl at admission were very strong predictors of conversion / failure of early laparoscopic cholecystectomy in acute cholecystitis.(7)

In another study on the impact of the Tokyo guidelines on the management of patients with acute calculouscholecystitisby Lee SW et al, they found out that lower levels of platelets, lower blood pressure, higher levels of C-reactive protein, blood urine nitrogen, prothrombin time, bilirubin, alkaline phosphatase, and more incidences of positive microorganisms cultured in bile or blood, were found in patients as the severity of disease progressed.(8)

In a study by Adamian et al, changes in the levels of plasma proteins albumin, transthyretine, transferrin, C-reactive protein, orosomucoid, and alpha 1-antitrypsin were followed up in patients with acute cholecystitis. The results in this study infer that acute-phase response develops in acute cholecystitis and its development is most accurately characterized by the concentrations of C-reactive protein. The content of this protein can be regarded as an indicator of the severity of inflammation of the gallbladder, and helps predict the disease course and define the terms of an intervention by the least invasive methods of treatment.(9)

Hepcidin is known as an acute phase protein. Hepcidin is expressed in the biliary epithelia and found in the bile where it is stress-inducible. The only study so far in relation to Hepcidin and acute cholecystitis is a study by PavelStrnad(10) which defined the expression and regulation of hepcidin within the biliary system; they showed that it represents an attractive marker reflecting the extent of the biliary inflammation (as shown for patients with cholecystitis and PSC-associated bacterial infection) and may play a role in the biliary response to bacterial infections and also concluded that further studies are warranted to analyze the potential of hepcidin as a biliary stress marker as well as to more directly evaluate its contribution to the defence against biliary infections. According to this study, the level of interleukin-6 inducible protein hepcidin is nearly 7 times higher in acute cholecystitis.(10)

There has been no study depicting the role of Hepcidin as a predictive or prognostic marker in acute cholecystitis, but the above study by Pavel Strand suggested that it could be a useful marker in acute cholecystitis.

Accumulating evidence indicates pathological roles for interleukin-6 in various disease conditions, such as inflammatory, autoimmune and malignant diseases. It has been found that Interleukin-6 is released in response to various bodily insults such as bacterial infection, tumor growth, cell injury, chemical irritants or ischemic necrosis mostly due to stimulatory effect of Interleukin-1 and Tumor Necrosis Factor-b. It then leads to various effects including release of interferon from B and T cells and fibroblasts, and acute phase response proteins such as C-reactive protein. The normal value for interleukin-6 in a healthy individual is expected to be <7 pg/ml.(11,12)

According to a study by J AViedma on acute phase reactants in relation to acute pancreatitis, endogenous inflammatory mediators have an important role in the pathogenesis of acute pancreatitis. Interleukin-6 concentrations tended to be higher in patients with necrotising pancreatitis and/or sepsis. The median value of Interleukin-6 was significantly different between the patients with 'mild' and 'severe' acute pancreatitis (p<0 001). The duration and the magnitude of raised serum concentrations of C-reactive protein are related to the extent of tissue injury.(13) There have been no studies so far on the role of Interleukin-6 in acute cholecystitis whether as a diagnostic or prognostic indicator, or as a predictor of severity.

With these limited studies available, our study attempts to correlate the levels of C-reactive protein, Hepcidin and Interleukin-6 with the severity of acute cholecystitis, the duration of complaints, and with the time for recovery of the patients.

From our study, we found that most of the cases of acute cholecystitis are of moderate severity when classified according to the Tokyo guidelines (43.9%).

The levels of C-reactive protein, Hepcidin and Interleukin-6 are increased in patients with acute cholecystitis. They are of diagnostic value in such patients.

The levels of C-Reactive protein, Hepcidin and Interleukin-6 correlate with the levels of total leukocyte counts at the time of admission (significance level {2-tailed} <0.01 for all the three markers), and with the duration of pain of acute cholecystitis before admission (significance level {2-tailed} <0.05 for all the three markers).

There was a significant correlation between the levels of these markers and severity of the disease. The mean levels of C-Reactive protein (mg/L) in mild / moderate / severe cases was 12.44 / 32.19 / 192.8, the mean levels of Interleukin-6 (pg/L) in mild / moderate / severe cases was 5.22 / 10.6 / 49.43 and the mean levels of Hepcidin(ng/L) in mild / moderate / severe cases was 27.52 / 43.06 / 108.06. This shows that as the severity increases the levels of these markers also correspondingly increase.

The elevated levels also correlate with the number of days of admission (p<0.001,) meaning that the period of recovery is longer if these values are higher and vice versa.

CONCLUSION

Based on the findings in our study, C-reactive protein, Interleukin-6 and Hepcidin levels evaluated at the time of admission of patients of suspected acute cholecystitis aids in the diagnoses of acute cholecystitis, assesses the severity of the disease, and will help predict the disease course and thereby help define the terms of an intervention by the least invasive methods of treatment.

LIMITATION AND RECOMMENDATION

Due to the relatively small number of patients, no definite guidelines for these markers as prognostic indicators can be drawn from this

study. However, a larger prospective trial done on the lines of this study could define the guidelines for prognostication of the patients with acute cholecystitis with the help of these markers.

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