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ORIGINAL ARTICLE

Evaluation of the Diagnostic Accuracy of Hepcidin and Conventional Biochemical Markers as Predictors in Disease Severity of Intra-Abdominal Inflammation

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Abstract

Introduction: Hepcidin is an iron-regulating protein that also behaves as an acute-phase reactant during the inflammation process. The aim of the current research was to assess the efficiency and usability of serum hepcidin levels in determining the severity of intraabdominal inflammation, and to compare hepcidin with other acute phase reactants.

Material and method: In a retrospective cohort, between December 2013 and January 2014 a total of 233 patients with acute cholecystitis and acute appendicitis were scanned at the emergency surgery clinic of Istanbul University, General Surgery Department, Istanbul Faculty of Medicine. After careful evaluation of exclusion criteria, 48 patients were enrolled in the study. Twice, on admission and the seventh day after diagnosis, blood levels of hepcidin and other conventional biochemical markers, and also abdominal examination findings were analyzed and compared.

Results: Twenty-eight of the 48 patients were men, and 20 were women. Eight patients were diagnosed having acute cholecystitis and 40 patients had acute appendicitis. The average blood analysis results at the first admission were C-reactive protein (CRP) 87.3 µg/dL (range, 1.8-430 µg/dL); white blood cell (WBC) 12.795 mcL (range, 5.300-24.500 mcL); ferritin 168.2 µg/L (range, 11.95-654.4 µg/L) $p = 0.004$; hepcidin 621.2 ng/mL (246-3274 ng/mL), and at the seventh day examination they were CRP 19.55 µg/dL (range, 1.2-131.3 µg/dL) $p < 0.001$, WBC 8694 mcL (range, 3400-13600 mcL) $p < 0.001$; ferritin 130.2 µg/dL (range, 10.3-563 µg/dL) $p = 0.004$; hepcidin 542.8 ng/mL (177-2800) $p < 0.001$.

Conclusion: Although CRP is more accurate in predicting the severity of intra-abdominal inflammation, hepcidin may be a useful biomarker for its sensitivity in the acute period of inflammation, thus helping the management of the disease.

Keywords

Hepcidin, C-reactive protein, Acute inflammation, Acute abdominal pain, Ferritin, Procalcitonin, White blood cells

Introduction

Acute abdomen is one of the most common clinical entities seen in emergency departments (ED). Abdominal symptoms occur because of localized or generalized intra-abdominal inflammation. Common biomarkers used for the diagnosis of intra-abdominal inflammation are white blood cell (WBC) counts, C-reactive protein (CRP), ferritin, and procalcitonin measurements. However, their specificity is low, because WBC or CRP levels tend to increase in all inflammatory diseases [1,2]. Several studies have explored the role of these biomarkers in improving the diagnosis of intra-abdominal inflammation in children and adults [3-8]. More accurate biomarkers are needed to reach the diagnosis for intra-abdominal inflammatory conditions.

Many microbial agents have been defined that prevent proteins from binding to the iron of the host. As a result, iron has a critical role in anti-microbial response [9]. The peptide hepcidin, which is mostly synthesized by hepatocytes in the liver, is the master regulator of iron homeostasis in vertebrates [10-13]. Some *in-vitro* studies have proven that hepcidin has an anti-microbial effect by increasing during inflammation [14].

In this prospective study, we aimed to assess the

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relationship of hepcidin with other biomarkers such as CRP, WBC, and ferritin in predicting the severity of intra-abdominal inflammation in the short and long term.

Material and Method

Between December 2013 and January 2014, a total 233 patients were diagnosed as having acute cholecystitis and acute appendicitis during and after their hospitalization period. After exclusions, 48 patients were enrolled in the study. This study was approved by the ethics committee of Istanbul Medical Faculty. Informed consent was obtained from all patients before taking samples and the study was conducted in accordance with the revised declaration of Helsinki.

Patients who presented to the emergency department with clinical acute abdominal pain of less than 48 hours duration and were clinically suspected by their general practitioners (GPs) of having acute appendicitis or acute cholecystitis were enrolled in the study. GPs based their clinical suspicion on several variables, including clinical status, disease history, and physical examination.

Patients who were excluded from the study were pregnant, those with comorbidities (hypertension, chronic obstructive lung disease, diabetes mellitus, chronic kidney disease and liver disease), and those with a history of abdominal trauma within 10 days of presentation. Patients with complications during post-op follow-up were also excluded from the study.

Patients were evaluated following a standard diagnostic procedure of history taking and physical examination. In addition to the routine laboratory tests, which were white blood cell counts and CRP measurements, hepcidin, ferritin, and albumin titers, were also measured at admission and at the seventh day examination. In accordance with the Optimization of Diagnostic Imaging Use in Patients with Acute Abdominal Pain (OPTIMA) study for acute abdominal symptoms, seven patients underwent surgery after imaging of the abdomen with ultrasonography (USG) and computerized tomography (CT). All patients who were diagnosed as having acute appendicitis and acute cholecystitis underwent surgery following their diagnostic examinations.

Hepcidin value count method

Blood samples were kept in a -80 °C environment until required for analysis. Hepcidin-25 levels were counted using solid phase enzyme-linked immunosorbent assay (ELISA) kits from (DRG International Inc, New York, ABD). The value range was accepted as 20-200 ng/mL. In addition to routine biochemical markers, 10 cc blood samples were drawn from each patient to detect hepcidin values and were also stored at -80 °C until required for analysis. Hepcidin values were measured in the biochemistry laboratory using ELISA analysis [15].

Plasma measurements

From each patient, an extra 5 mL of blood was collected in an EDTA tube (BD Vacutainer, Becton Dickinson Diagnostics, Breda, the Netherlands) in the ED. These samples were immediately centrifuged after collection for 12 minutes at 2100 rpm and cooled to 5 °C. Laboratory technicians then directly removed the plasma portion from each EDTA tube and transferred it into multiple cryopreservation tubes and stored at -20 °C until required for analysis [16].

Samples were assayed in accordance with the manufacturer's recommended procedures by trained laboratory technicians in the specialized laboratory facilities of the Department of General Surgery. The laboratory personnel were unaware of the final diagnosis and of the C-reactive protein, WBC, ferritin, procalcitonin, and albumin values. Samples were run in duplicate, and a variability of 5% between sample duplicates was accepted. Values at admission and from the seventh day were recorded. The mean and median values were recorded later for each biomarker. Concentrations were determined in a standard fashion by the laboratory of clinical chemistry and hematology.

Hepcidin values were compared with WBC, CRP, procalcitonin, albumin, and ferritin values according to the different age groups. Hepcidin values were counted on the fifth or seventh day of the healing period.

Statistical analysis

Demographic properties, the average value calculated on the first admission for CRP, WBC, ferritin, procalcitonin, albumin, hepcidin, and at the seventh day examination, physical examination findings, treatment plans, hospitalization, and complications were recorded in a database. All statistical analyses were performed using the Statistical Package for the Social Sciences version 21.0 (SPSS, Inc, Chicago, IL, USA). The standard deviation of continuous variables was calculated using the Shapiro-Wilk test. Independent samples test and Mann-Whitney test were used for the two-group comparison of continuous variables. A P value of < 0.05 was considered statistically significant.

Results

A total of 233 consecutive cases who were seen in the ED with suspected acute appendicitis and acute cholecystitis (101 males, 132 females; mean age = 30.3 years; range, 5-83 years) were evaluated. Of these, 48 patients were enrolled in the study, 8 of which had acute cholecystitis and 40 had acute appendicitis. The average age of the patients was 34.4 years (range, 18-78 years). The average time of hospital stay was 2.125 days (range, 1-6 days). All of the patients who were diagnosed as having acute appendicitis or acute cholecystitis underwent surgery. No complications were recorded in the post-op period (Table 1 and Table 2).

Table 1: Laboratory findings are given at admission and the seventh day examination. There was a high percentage of statistical significance.

	Values at admission		Seventh day examination		
	mean ± s.d	Median (min-max)	mean ± s.d	Median (min-max)	P
WBC	12.795 ± 4360	12.550 (5300-24500)	8.694 ± 2495	8.450 (3.400-13.600)	< 0.001
CRP	87 ± 111	41 (2-430)	20 ± 27	11 (1-132)	< 0.001
Ferritin	168.3 ± 145.7	127.4 (12-654.4)	130.2 ± 108.3	107.4 (10.4-563.9)	0.004
Procalcitonin	0.4 ± 0.5	0.2 (0-2.7)	0.2 ± 0.3	0.1 (0-1.3)	< 0.001
Albumin	3.8 ± 0.5	3.7 (2.7-4.9)	3.5 ± 0.5	3.6 (2.8-4.8)	0.001
Hepcidin	621.270 ± 571.943	371.20 (246.531-3274.716)	542.832 ± 471.581	371.685 (177.250-2800.720)	< 0.001

Table 2: Measurement of two different conditions is given. Based on the p values, the results for acute appendicitis appear to be more accurate.

	Acute cholecystitis					Acute appendicitis				
	Mean ± SD	Median	Min	Max	P	Mean ± SD	Median	Min	Max	P
CRP	113 ± 98	46	26	248	0.018	83 ± 113	37	2	430	< 0.001
C. CRP	30 ± 46	12	5	132		18 ± 23	10	1	92	
WBC	12.500 ± 2908	12.300	8.300	17.500	0.128	12.845 ± 4588	12.700	5.300	24.500	< 0.001
C. WBC	8986 ± 2538	9600	4300	12300		8644 ± 2516	8300	3400	13600	
Ferritin	222.1 ± 210.3	168.4	12.0	654.4	0.176	159.1 ± 133.1	126.2	22.1	568.6	0.009
C. Ferritin	171.2 ± 205.4	82.8	10.4	563.9		123.2 ± 84.3	110.8	15.1	368.1	
Procalcitonin	0.3 ± 0.2	0.3	0.1	0.7	0.063	0.4 ± 0.5	0.2	0.0	2.7	< 0.001
C. Procalcitonin	0.2 ± 0.2	0.2	0.0	0.5		0.2 ± 0.3	0.1	0.0	1.3	
Albumin	3.9 ± 0.5	4.1	3.2	4.6	0.034	3.7 ± 0.5	3.7	2.7	4.9	0.004
C. Albumin	3.4 ± 0.4	3.3	2.9	4.1		3.6 ± 0.5	3.6	2.8	4.8	
Hepcidin	400.97 ± 145.24	356.88	284.31	651.54	0.176	658.88 ± 609.31	378.49	246.53	3274.72	< 0.001
C. Hepcidin	368.83 ± 106.09	325.25	250.12	521.35		572.54 ± 503.4	378.25	177.25	2800.72	

Discussion

Biochemical markers are needed to determine the severity of intra-abdominal inflammation. To improve the diagnostic accuracy for intra-abdominal inflammation, several studies have focused on plasma markers; hepcidin is one of the most recent that has been investigated. Like other conventional biomarkers such as C-reactive protein, white blood cells and procalcitonin, the pattern of hepcidin protein shows a correlation with the inflammation process. There is still no consensus on the gold standard method for clinical use [17,18]. Signs of acute inflammation, including an increase in WBC count, frequency of neutrophils, and increase in CRP levels were significantly correlated with the hepcidin levels. The inflammation process can be initiated by bacterial infection, surgical stress, and inflammatory cytokines, interleukin 6 (IL-6) induces the expression of hepcidin [19].

The vast majority of iron is associated with proteins in biologic systems [20]. Although located in the structure of certain proteins such as hemoglobin and myoglobin, iron is also found in the structure of some critical enzymes required for oxidative phosphorylation. As such, all of these make iron very fundamental. Hepcidin concentrations increase in inflammation. However, even in patients without significant inflammation, it can be seen that hepcidin levels elevate progressively with the severity of a disorder [21].

All patients who are admitted with abdominal pain should be first questioned about the history of pain, i.e. how and when the symptoms began. Then a detailed physical examination should be performed. Imaging techniques and laboratory tests can help the physician for the diagnosis of acute abdomen. However, none of these methods are more valuable than the physical examination. In addition, unnecessary imaging modalities may cause negative cost-effectiveness results and also the high doses of radiation given to the patient may have serious adverse effects in the future [22,23]. All of these entities have high significance because morbidity and mortality rates can increase owing to misdiagnosis of acute abdomen. Thus, we preferred hepcidin measurement to provide greater accuracy.

Hepcidin levels increase during acute abdominal inflammation and also decrease during the healing process of the post-op period [24]. Kyung Hwan Park, et al. showed in a study of abdominal surgery that postoperative inflammation increased the production of hepcidin after surgery in the acute period [25]. As it was in our current study, the average hepcidin levels calculated at admission was 653.8 ng/mL (range, 364-3274 ng/mL) and was 536.2 ng/mL (range, 315-2800 ng/mL) at the seventh day examination; the difference was statistically significant ($p < 0.001$).

CRP and WBC are the most commonly used biomarkers for estimating the severity of inflammation because of their high sensitivity. CRP, which has an average half-life of 19 hours, reaches it maximum level at 48 hours and shows a close correlation with ongoing inflammation [26]. In our study, the peak values of CRP and WBC were measured at 48 hours and then decreased dramatically throughout the hospitalization period, which

closely correlated with the clinical condition of the patients. The mean values calculated for CRP and WBC at admission were 87.6 µg/dL and 12795 mcL, and at the seventh day follow-up were 20 mcL and 8694 µg/dL, respectively ($p < 0.001$). These results showed high accuracy and a close correlation with the clinical process.

Nevertheless, no relationship was found with hepcidin and the other conventional biomarkers. Unlike, CRP, did not show the same correlation with other acute-phase reactants like procalcitonin, leucocytes and ferritin during the early and late phase of inflammation, which makes CRP a more sensitive biomarker.

Conclusion

Hepcidin is a new, safe, biochemical marker that was helpful in determining the severity of intra-abdominal inflammation when compared with routine biomarkers. Although there are few studies in the literature showing the role of hepcidin in determining inflammation, monitoring hepcidin levels could provide diagnostic support in the management of acute abdomen. We believe that hepcidin could be as useful as other acute-phase reactants, but more studies are needed to accurately identify its role.

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