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ORIGINAL ARTICLE

Biochemical Study for Inflammatory Proteins and Some Enzymes in Sera of Iraqi Patients with Gastrointestinal Obstruction

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ABSTRACT
This study attempted to evaluate the association of some inflammatory proteins and enzymes with the risk of GIT obstructions patients. About (40) volunteers participated in this clinical evaluative study aged between 30 to 50 years. Total serum protein and albumin levels, alkaline phosphatase , α-amylase activities were measured by spectrophotometric methods. Acute phase protein high sensitive C reactive protein(hs-CRP) and interlukein-10(IL-10) were measured by a sensitive sandwich ELISA kit (HUMAN,Germany). Analysis of the results showed highly significant (P < 0.001) differences in the activity and specific activity of ALP and α-amylase in sera of patients compared to control group. Also an increased in the levels of many inflammatory proteins, The results have shown that serum hs-CRP,IL-10 are elevated when GIT tissues are impaired due to its obstruction . According to CRP findings we suggest that CRP,hs-CRP may have a clinical utility as a biomarker in serum and should be included in the routine laboratory work-up for risk evaluation and stratification in causes of GIT obstruction due to presence the stones without an inflammation .
Keywords: *Acute phase protein, hs-CRP, IL-10 , ALP ,Amylase, Gastrointestinal obstruction.*

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INTRODUCTION

Gastrointestinal (GI) obstruction may be due to inflammatory bowel disease (inflammatory bowel disease; Crohn's disease, ulcerative colitis and indeterminate colitis), malignancy (colorectal cancer)[1], Inflammatory bowel disease (IBD) refers to two chronic diseases that cause inflammation of the intestines: ulcerative colitis (UC) and Crohn's disease (CD) that are immune mediated, multifactorial,chronic, relapsing and remitting[2].

Serological markers include C-reactive protein, erythrocyte sedimentation rate and antibodies, Faecal markers that can aid in distinguishing inflammatory disorders from non-inflammatory conditions are non-invasive and generally acceptable to the patient [1].There is substantial evidence that immunologic factors play a role in the pathogenesis of GIT disease .The alteration in cytokines level and other angiogenic factors may explain the adhesion, implantation and the progression of the transported fragment of tissue in different site of the gastrointestinal organs. Many cytokines have been studied to prove the immunological theory of many GI disease, not abstraction .Bowel obstruction may be mechanical or functional, partial or complete, and may occur at one or at many sites. Tumors can impair bowel function in several ways [3-4].

Early complications associated with percutaneous endoscopic gastrostomy are well documented. Late complications associated with retained gastrostomy flange are rare. It is unclear why some patients with retained gastrostomy flange (internal bumper) develop mechanical obstruction and others do not[5].

Alkaline phosphatase (ALP) is present in a number of tissues including liver, bone, intestine, and placenta. The activity of ALP found in serum is a composite of isoenzymes from those sites and, in some circumstances, placental or Regan isoenzymes. Serum ALP is of interest in the diagnosis of 2 main groups of conditions-hepatobiliary disease and bone disease .A rise in ALP activity occurs with all forms of cholestasis, particularly with obstructive jaundice. The response of the liver to any form of biliary tree

obstruction is to synthesize more ALP. The main site of new enzyme synthesis is the hepatocytes adjacent to the biliary canaliculi. Thus the serum alkaline phosphatase is a measure of the integrity of the hepatobiliary system (liver, bile duct, or gallbladder dysfunction)and the flow of bile into the small intestine.It has often been measured as possible indicators of GIT inflammation and bone metabolism, alkaline phosphatase levels change in relation to GIT inflammation and bone loss.[6].The serum alkaline phosphatase is a measure of the integrity of the hepatobiliary system and the flow of bile into the small intestine. An increased serum Alkaline Phosphatase may be due to: Congestion or obstruction of the biliary tract, which may occur within the liver, gallbladder, pancreas, or duodenum[7].The pancreas is a gland that is situated behind the stomach and that releases hormones and enzymes to help the body break down and absorb nutrients from food. Amylase is a pancreatic enzyme that is secreted during the digestion process to convert starches to sugars. Amylase test use to diagnose pancreatic inflammation, commonly known as pancreatitis. Cholecystitis, intestinal blockage, acute pancreatitis and certain medications can cause higher-than-normal amylase levels. A blockage of the bile duct is the most common cause of gallbladder inflammation Most episodes typically last a few days, after which amylase levels usually return to normal[8].

Cytokines are soluble messenger proteins produced by a whole range of different cell types which exert their actions within a local environment or in a systemic manner to modify and regulate immunological and inflammatory reactions as part of their response[9,10]. Cytokines are classified into sub groups as(growth factors, interleukins, interferons and miscellaneous).

The levels of liver protein change during inflammation are termed acute phase proteins (APPs) Acute-phase proteins (APPs) are an evolutionarily conserved family of proteins produced mainly in the liver in response to infection and inflammation[12]. Interleukins and tumor necrosis factor (TNF) are the major cytokines that stimulate the liver to synthesize(c-reactive protein) C-RP and other positive acute-phase proteins. There are large and varied groups of glycoproteins in serum released into blood stream in response to a variety of stress. All the up-regulated proteins have been called positive APP like C-reactive protein(C-RP), in order to differentiate them from the so-called negative APP that is down-regulated like albumin[13]. Another biomarker often used in the emergency department to aid in the diagnosis of an acute abdomen is the C-reactive protein (CRP). Most studies have focused mainly on the use of this parameter in establishing the diagnosis of appendicitis. Few studies have assessed its diagnostic role in the general conditions describing acute abdominal pain. The diagnostic value of CRP in the overall patient with acute abdominal pain showed a sensitivity of 79%, specificity of 64% and global accuracy of 73% for predicting subsequent hospitalization using a cut-off value for positive test of >5 mg/L. More recently, Salem et. al. [14] reviewed the diagnostic value of CRP in true surgical patients with acute abdominal pain in the ED. They concluded that CRP alone is not useful in differentiating between surgical causes of acute abdomen or self-limiting condition. In addition, CRP can neither differentiate between surgical conditions requiring intervention from those who can be treated non-operatively . In conclusion, these studies confirm the difficulty to diagnose an acute abdomen and assessing the need for a laparotomy as in our cases. Although high CRP levels or increase in CRP concentrations are seen in combination with abdominal complaints, it does not directly mean that a surgical complication should be the problem[14].

SUBJECTS AND METHODS

Samples were collected from patients among the Gastro Intestinal Center –Teaching Baghdad Hospital/Health Ministry of Iraqi during the period from January to April 2014.A total of 40 subjects were collected in this study, 20 cases injured with different GIT obstructions(11in stomach,6 in bile tract ,in 3 in small intestinal) age between (35-50) years. All of them were selected randomly. A total of 20 healthy volunteer's age between (35-50) years served as a control group was also included in the study. The study was excluded all subjects who smoking, alcohol drinking, taking antibiotic drugs, as well as individuals suffering from chronic or acute disease (e.g. diabetes mellitus, hypertension, renal disease

SPECIMEN COLLECTION

Five milliliters (5ml) venous blood was collected from the patients and healthy men and women .Blood samples were left for 20 minutes at room temperature. After coagulation, the sera were separated by centrifugation at (1500xg) for 10 min after collection, hemolysed samples were discarded. The sera was stored at (-20) ºC until use for different investigations. The samples were not thawed and refrozen before testing.

METHODS

Total serum protein (T.S.P), albumin ,and alkaline phosphatase ,α-amylase activities were measured by spectrophotometric methods supplied by Human Diagnostic, Germany .Specific activities(U/mg) for each enzyme were calculated by divided the activity on the total amount of protein . Globulin concentration

and [albumin]/[globulin] ratio in sera samples of the studied groups in this study were calculated. Acute phase protein high sensitive C reactive protein(hs-CRP) and interlukein-10(IL-10)were measured using commercially available Enzyme Linked Immunosorbent Assay (hs-CRP and IL-10 : biocheck, Company, USA**.**

STATISTICAL ANALYSIS

The data collected were analyzed using statistical package for social science (SPSS version 15.0). Percentage prevalence rates were calculated with their respective 95% confidence intervals. Differences between proportions were evaluated using T- tests & Mann-Whitney U test Statistical significance were achieved at $p < 0.05$.

RESULTS AND DISCUSSION

The mean **±**SD of ALP & α-amylase activities and their specific activities measured by (U/L)&(U/g) units respectively, in serum of GIT obstruction patients and control groups are illustrated in Table (1)**.** The results showed that ALP &α-amylase activities levels were higher in patients than in control groups , while their specific activities were otherwise, lower for the same groups. The activities of alkaline phosphatase (ALP) and α- amylase were increased with highly significant ($p \leq 0.001$) for patients in compare to control groups. This result was similar to that obtained by some studies which explained that both of them are GITs enzymes and two ALP isozymes presents in liver, small intestinal while amylase in pancreas gland and saliva therefore any injuring or inflammation in their tissues (obstruction or tumor) would releasing the enzymes from the tissue to the blood[15,16].ALP elevations tend to be more marked (more than 3-fold) in extra hepatic biliary obstructions (eg, by stone or cancer). With obstruction, serum ALP activities may reach 10 to 12 times the upper limit of normal [17].

The observation that ALP & α-amylase were tested in this study to show whether obstruction in GIT had an effect on these enzymes. It was observed that ALP had an increased above the normal values when compared to its values in normal individuals. The present findings are in agreement with AL-Atrakchi S.A.[18]. In humans, the enzyme ALP is found throughout the body in the form of isoenzymes, deriving from not only the liver and bone, but also intestines, placenta, kidneys, and leukocytes[19].

Table (1): Mean values of patients and control subjects according to ALP & α- Amylase

Parameters	Patients				Control
	Minimum	Maximum	Mean ± SD	SE	Mean ± SD
ALP activity (U/L)	63.87	1114.71	269.7318±239.860	3.634	189.0±100.0
ALP specific activity (U/g)	0.48	6.26	2.1395±1.5642	0.3497	2.7±1.0
α-Amylase activity (U/L)	0.1	910.06	306.1374±291.805	65.2417	220.0±110.0
Amylase specific activity (U/g)	0.0	7.455	2.8663±2.6008	0.5815	2.97±0.87

Table (2): Mean values of patients and control subjects according to total protein, albumin, globulin, hsC.R.P and IL-10.

Acute phase proteins	Control Mean± SD No.(20)	gastrointestinal Obstruction Mean± SD No.(20)	P- value
Total protein (g/dL)	8.04±1.9277	11.19±2.3753	0.000
Albumin (g/dL)	3.7413±0.7308	2.044±0.7914	0.000
Globulin (g/dL)	4.2987±1.1969	9.146±1.5839	0.000
Albumine/Globulin	0.8703±0.6105	0.2234±0.4996	0.000
hs C.R.P (µg/ml)	8.7079±12.105	23.6383±21.4050	0.021
IL-10 (pg/ml)	0.82±0.2251	1.4333±0.9656	0.014

Serum ALP is of interest in the diagnosis of 2 main groups of conditions-hepatobiliary disease and bone disease associated with increased osteoblastic activity. A rise in ALP activity occurs with all forms of cholestasis, particularly with obstructive jaundice. The response of the liver to any form of biliary tree obstruction is to synthesize more ALP [17]. By now, it is not clear whether the observations made for

serum ALP in GIT patients mainly reflect changes in bone and mineral metabolism or other systemic processes [19].

The intestines are designed to move food through the digestive tract and absorb nutrients. Undigested food and digestive fluids can become trapped in the intestines when an obstruction occurs, causing high amylase levels. A test to measure amylase levels is sometimes used to monitor pancreatic cancer treatment. Amylase levels might remain elevated until the cancer is under control [20].Sometimes; high amylase levels are not associated with a particular disease or condition. Certain drugs such as aspirin, codeine, furthermore consuming alcohol therefore all persons in this study had recommend avoiding the consumption of alcohol or any drugs.

The mean levels of total proteins, albumin, globulin and acute phase proteins (hs.C.R.P. & IL-10) were illustrated in table (2) for both groups patients & control .All the mean values (except albumin with its ratio) had elevated with highly significant ($p \leq 0.001$) and significant increase ($p \leq 0.05$) for hs-C.R.P & IL-10 for all patients with gastrointestinal obstruction in compare to control groups .The above data are confirmed by Liao S.W.*et al*., results who established that acute inflammation causes relative reduce in albumin synthesis in the liver then decreasing its level secreted to the blood[21]. Also Snyder CW *et al*., evaluated the association of early hypoalbumine-mia with the risk of intestinal failure in gastroschisis patients .They concluded that early severe hypoalbuminemia was strongly associated with intestinal failure[22].While, Herczeg B., investigated the effect of a simple, low intestinal obstruction in dogs on the leakage of 131I-serum albumin from the circulation into the intestine. An increase leakage has been demonstrated. The increase in albumin leakage observed during intestinal obstruction resulted in 33% rise of total catabolism[23].Dhruva K.G. *et al*., reported that some cytokines and IL-10 altered synthesis protein s liver. Then increasing CRP formation which called (positive reactive proteins) and decreasing albumin e levels for patients with GIT obstruction especially those caused by presences a stone and not an inflammation, then albumin level could be used as indicator for gastro or intestinal inflammations[24]. In the current study, patients with GIT inflammation or tumors had a positive results for the Rapid Test of CRP while, the other patients with GIT obstruction due to s had negative results for Rapid Test of CRP.Gavela T,*et al*., predict stoned the outcome of pediatric patients with appendicitis,that children with CRP greater than 3 mg/dL and/or PCT(procalcitonin test) greater than 0.18 ng/mL have a greater risk of complications; thus, intervention should be early, and patients should be monitored closely[25].

Interleukin-6 (IL-6) is a multi-functional cytokine that regulates immune responses, acute phase reactions and may play a central role in host defense mechanisms [26]. Interleukin-10(IL-10) expressed the equilibrium status between all other interleukins and the main body defenses' mechanisms ,the Mean± SD of IL-10 in Table-2 obvious a significant increase ($P \leq 0.05$) for patients in compare to control group ,this was in agreement with Todoro Yic T *et al*.,[27] who showed that the increasing in these markers concentrations expressed an inflammation responses and the intensity of this response related with the amount of these markers increased [28]. whereas there was only a mild increase in the anti-inflammatory cytokine IL-10, suggesting an imbalance of cytokine production favoring the pro-inflammatory response[29-31].Recombinant human IL-10 has been produced and is currently being tested in clinical trials. This includes rheumatoid arthritis, inflammatory bowel disease, psoriasis, organ transplantation, and chronic hepatitis C[32].

In conclusion, these results have shown that serum hs-CRP,IL-10 are elevated when GIT tissues are impaired due to their obstruction . According to CRP findings we suggest that CRP may have a clinical utility as a biomarker in serum and should be included in the routine laboratory work-up for risk evaluation and stratification in causes of GIT obstruction

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