Enzymes and other Laboratory Tests in Human Pancreatitis

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الخلاصة:

أجريت الدراسة السريرية لعشرين مريضا مصابا بالتهاب البنكرياس، تراوحت أعمارهم ما بين (٤٥-٦٨) سنة، ممن يراجعون مستشفى الزهراوي في مدينة الموصل، وتم إجراء التقييم السريري والمختبري لكل مريض بالتهاب البنكرياس ، اذ شملت الفحوصات المختبرية قياس مستويات الصوديوم والبوتاسيوم والمغنيسيوم والكالسيوم والالبومين والكليسيريدات الثلاثية والكلوكوز واليوريا والبيليروبين الكلي وانزيمات الامايليز ولاكتيت ديهيدروجينيز واسبارتيت ترانس امينيز.

أوضحت نتائج التحليل الإحصائي إن مستوى (الصوديوم، الكالسيوم، الالبومين، البوتاسيوم والمغنيسيوم) في مصل الدم قد انخفض بشكل معنوي واضح عند مرضى التهاب البنكرياس مقارنة مع مجموعة السيطرة ، في حين إن مستوى (الكليسيريدات الثلاثية، الكلوكوز، اليوريا، البيليروبين الكلي، لاكتيت ديهيدروجينيز، اسبارتيت ترانس امينيز والامايليز) أظهرت ارتفاعاً معنوياً عند مرضى التهاب البنكرياس مقارنة مع مجموعة السيطرة.

Abstract:

A clinical study of twenty patients with pancreatitis, aged between (45-68) years, attending Alzahrawi hospital in Mosul city.

Each pancreatitis patient was evaluated clinically and laboratory examinations have been done including: (Serum sodium, potassium, magnesium, calcium, albumin, triglycerides (T.G), glucose, urea, total bilirubin, amylase, lactate dehydrogenase (LDH, Aspartate transamines (AST).

The analysis of results showed that the levels of Serum Sodium, Calcium, Albumin, Potassium and Magnesium were significantly decreased in pancreatitis patients when compared with control, while T.G, glucose, urea, total bilirubin, LDH, AST and amylase levels were

significantly elevated in pancreatitis patients when compared with control.

Introduction:

Pancreatitis is a potentially serious disorder characterized by inflammation of the pancreas that may cause autodigestion of the organ by its own enzymes. This disease has two manifestations: acute and chronic pancreatitis [(1)].

Acute pancreatitis (AP) is the result of an inflammatory process involving the pancreas caused by the release of activated pancreatic enzymes.

In addition to the pancreas, this disorder can also affect surrounding organs, as well as cause a systemic reaction. While this form of the disease resolves both clinically and histologically, approximately 15% of patients with AP will develop to chronic pancreatitis [(1,2)].

In clinical terms, however, AP is often defined as abdominal pain associated with hyperamylasaemia of greater than three times above normal [(3,4)]. Serum amylase concentration, useful for early diagnosis, provide little correlation with disease severity [(5)].

AP affects a round 40/100.000 of the Western general population, and its attacks are classified as severe in 20-30% of patients [(6,7,8)].

Chronic pancreatitis (CP) develops from chronic inflammation of the pancreas that results in irreversible and progressive histological changes. This includes fibrosis and ductal structures, which destroy the pancreas directly, as well as decreased endocrine and exocrine functions, which can negatively affect other body systems [(1,2)].

The pathophysilogy of pancreatitis remains poorly understood. The main causes of pancreatitis in adults are hypertriglyceridemia, Gallstones and alcohol consumption [(1,9)].Because AP results in the release of pancreatic enzymes from injured ancinar cells, an increase in serum enzymatic levels is key to diagnosing this disorder. While pancreatic digestive enzymes normally do not become active until they reach the small intestine, pancreatitis causes the unwarranted activation of trypsinogen within ancinar cells of pancreas, which is then converted into the enzyme trypsin. Large quantities of trypsinogen are converted to trypsin so that the normal regulation and removal of trypsin from the cells can not adequately maintain the proper balance. Therefore, because the activation of these enzymes creates a build up and may lead to pancreatic autodigestion [(10)].

Other tests suggestive of AP include hypertriglyceridemia and an increase of bilirubin in

(15 - 25%) of patients because pancreatic edema compresses the common bile duct [(1,2)]. Bilirubin may only be abnormal if there is considerable bile duct compression from a pseudocyst or fibrosis.

Liver enzymes may be elevated in patients with alcoholic liver disease. Elevated AST activity is associated with gallstone pancreatitis, threefold elevation or greater in the presence of AP has a positive predictive value of 95% in diagnosing acute gallstone pancreatitis [(11)].

Low levels of serum albumin, is a late marker, associated with pancreatic necrosis may be due to fluid and protein third-space losses.

Pancreatitis can create systemic complications. Damage to the pancreas can disrupt normal operation, in particular its endocrine and exocrine functions. One part of the pancreas makes insulin and glucagon (the hormones that control blood sugar level), and the other part makes enzymes that help small intestine to break down the fatty acid and polypeptide in smaller parts. Blood tests like blood glucose will help to monitor over all condition and watch for complications. Beta cells within the pancreas serve the endocrine function by secreting insulin and glucagon directly into the blood to help the body to regulate glucose, and when these cells are destroyed diabetes mellitus may occur [(2,10,12)].

Patients are also at risk for hyperglycemia because of the possible damage of islet cells in the pancreas and may need insulin to combat consistently elevated blood glucose levels. However, the most profound effects chronic pancreatitis can have are: maldigestion of fat and decreased insulin and glucagon production. Together, these complications can result in the development of diabetes mellitus[(1)]. Patients who develop DM require insulin to control their blood sugar, so that, oral pancreatic enzyme supplements are often given with every meal to help patients digest food if the pancreas does not secrete enough enzymes on its own[(13)].

Patients generally require vigorous fluid replacement to prevent hypovolemia caused by third-space losses and vomting, usually with a colloid or Ringer`s lactate solution. Brank[(14)] indicates that increase blood urea nitrogen (BUN) may indicate hypovolemia, patients are at risk for decreased renal perfusion and acute renal failure due to hypovolemia. The glomerular filtration rate drops with hypovolemia, and induces the release of renin, which constricts blood vessels and elevate, aldosterone levels, decreasing renal perfusion.

A recent study[(15)] indicates AP by elevation in serum amylase and LDH activities.

Rau et al[(16)] investigated serum LDH levels daily in 70 patients with AP, the researchers found that LDH provided good discrimination for AP.

Electrolyte imbalances can be potentially life thereatening for patients with pancreatitis. Hypokaemia, hypocalcemia and hypomagnes-emia are the most frequently seen electrolyte imbalances. Hypocalcemia is often associated with severe pancreatitis and persistently low levels increase mortality. Also patients with pancreatitis are at risk for

hypokalemia duo to prolonged vomiting or the loss of potassium with protein-rich fluid that leaks into the peritoneal cavity [17].

This study discusses the role of biochemical tests in establishing the diagnosis of pancreatitis, predicting its severity and determining its cause.

Materials and Methods:

A clinical study of 20 patients with pancreatitis attending Alzahrawi hospital in Mosul city, was conducated. A control group, consisting of ten healthy subjects were used. Clinical laboratory examination on serum including, Na^+, K^+, Ca^{++}, Mg^{++}, Albumin, glucose, urea, T.G., total bilirubin, AST, LDH and serum amylase levels were evaluated. We used phadebas amylase test from pharmacia, Uppsala, Sweden to measure serum amylase activity [18]. Serum Na^+ and K^+ were measured using flame photometry [19]. While serum AST was determined by a colorimetric method [20]. Calcium was determined colorimetrically without deproteinization using o-cresol phthalein complexone interference due to Mg^{+2} ions is eliminated by 8-hydroxy quinoline up to 4 mmol/L (10mg/100ml or 100mg/L) [21].

Magnesium determined by atomic absorption spectrometry technique [22]. Triglycerides levels were determined colorimetrically [23]. Serum albumin was determined by bromocresol green (BCG) dye binding method [24]. While the levels of serum total bilirubin were based on the reaction of bilirubin with diazotized sulfanilic acid (Diazo method) [25]. Serum glucose was assayed using enzymatic method [26]. Serum urea was determined using diacetyl monoxime method [25]. LDH was measured with Boehringer Mannheim kit according to established method and results expressed in IU/L [26]. The upper limit for the normal serum LDH value in our method during the study was 200IU/L.

Statistical analysis have been done and all data have been expressed as mean ± standard diviation. Student's t-test [27] was performed for evaluation of significant differences between groups differences were assumed to be significant when $p \leq 0.05$.

Results and disscusion:

Serum amylase remains the most commonly used biochemical marker for the diagnosis of acute pancreatitis, but its sensitivity can be reduced by late presentation, hypertriglyceridaemia, and chronic alcoholism [28,29].

A raised level of serum amylase activity, at least three time the upper limit of normal, supports the diagnosis of AP [30,31].

Hypertriglyceridaemia competitively interferes with the amylase assay and can produce falsely low results [31].

This mean that low s.amylase values always eliminated the possibility of pancreatitis and the high values often, but not always, diagnostic for pancreatitis [30]. Severe hypertriglyceridaemia may occur at 20% of patients with AP [32]. This may be occur because the TG rich lipoproteins interfere with the amylase estimation, although a circulating inhilaitor may also have an effect [33]. Also low serum sodium concentration should alert clinicians to the possibility of severe hypertriglyceridaemia [34]. When a pancreatic duct gets blocked for whatever reason, the pancreatic juices which contain digestive enzymes will not drain properly into the duodenum. A build up of this stuff causes auto-digestion. Important biochemical markers for AP are s.amylase, LDH, AST, TG, Na^{+}, K^{+}, Ca^{++}, Mg^{++}, Albumin, glucose, urea and total bilirubin levels. Both acute and chronic pancreatitis may be diagnosed by blood tests that measure pancreatic function. In AP amylase spills into the blood stream, so that its level is markedly elevated. Raised levels of other important chemicals such as TG, glucose, urea, total bilirubin, LDH and AST can also be detected. Over a period of times CP can lead to DM, calcium deficiencies or infections of the pancreas [35].

Normally, digestive enzymes secreted by the pancreas do not become active until they reach the small intestine. But when the pancreas is inflamed, the enzymes inside it attack and damage the tissues that produce them. Insulin is the most important hormone that is produces by the pancreas because it regulate the levels of glucose in the blood. AP patients may have high amounts of sugar and lipids, or fat, in their blood, these changes help the doctor to diagnose pancreatitis [36].

AP complications include development of pancreatic pseudocyts, pseudocysts may press on and block structures such as the bile duct, thereby leading to elevate bilirubin levels and may be cause jaundice [37].

(The Table) showed elevation in total bilirubin in AP patient group, perhaps due to mild biary obstruction by pancreatic edema or the passage of studge (or small stones)[38]. Or may caused by pancreatic disease which raised conjugated bilirubin levels [39].

The typical liver panel will include blood levels of enzymes found primanly from the liver (such as AST) and protein levels, specifically albumin; and some bone (Ca^{++}) and heart (LDH) disorders can lead to an increase in aminotransferases. AST levels greater than 10x tends to indicate acute hepatocellular, less than this tend to indicate obstructive causes [40].

The results in this study showed a significant differences in AST, LDH in pancreatic patients group and control group as seen in the table, and this study a grees with the results of previous studies [41,42]. It has been established that abnormalities in blood concentrations of AST on admission with AP may be applied to indicate a biliary etiology [43].

Davidson et al [44] reported that an elevation of serum AST predicted gallstone related attacks with sensitivity. In another study, Grau et al [45] found that an elevation of AST of 1.2 times above the upper limit of normal range within the initial 24 hours of AP.

LDH is an enzyme that is found in almost all body tissues,but only a small amount of it is usually detectable in the blood. Contained within the tissues cells, LDH is released into the blood stream when cells are damaged or destroyed. Because of this the LDH test can be used as a general marker of injury of cells, but the dynamic of LDH in the AP is not well studied. As shown in the table, serum urea concentrations are significantly higher in patients group as in control, which is consistent with the finding of other investigators, like Bechien et al [46] which found that blood urea nitrogen (BUN) levels were persistently higher in AP patients vs. normal control. BUN is waste product filtered out the blood by the kidneys. Increased concentrations in the blood may indicate a temporary or chronic decrease in kidney function.

Also the table shows hyponatremia and hypokalemia in patients group as compared with control group. This finding differs from the results of the previous studies [47,48] in which pancreatitis often associated with hyponatremia and normokalemic. However, our results were similar to the report from others [48].

Sodium and potassium are the major cations found in the pancreatic fluid at the concentrations similar to the extracellular fluid levels. Normal plasma Na^+ and K^+ concentrations are maintained by balanced intake and excretion, intra cellular and extracellular osmotic pressure, and pH [49]. Hyponatremia is primarily associated with renal sodium wasting and water retention due to an ability to excrete ingested water [50].

Hypokalemia may result from both a decrease shift of the ion from the intracellular to the extracellular compartment and an increase in the renal excretion of potassium. It is particularly important that the signs and symptoms of changes in plasma K^+ concentrations should be particularly recognized and quickly treated, because the changes are potentially life threatening [51,49]. Decreased K^+ also occurs in diarrhea and vomiting which are associated with pancreas injury.

In our study we reported changes in the serum calcium and magnesium (Table 1). Hypocalcemia is less commonly observed in pancreatitis and is a frequent complication of feline AP. Matul [52] found that lower concentrations of calcium have been associated with a poor diagnosis of AP. While others[53] found that hypocalcemia in experimental pancreatitis occurs in dependently of changes in serum nonesterified fatty acid levels.

Table(1)shows low levels of serum calcium in patients group as compared to control, this results is with agreement with those found by[54]

which indicate that plasma calcium concentration decreased in cats with AP. The calcium homeostatic system depends on several important factors: parathyroid hormone (PTH), vitamin D, phosphate, and magnesium [(49)]. PTH plays important role of s-calcium by its action on the kidney. It increases the tubular reabsorption of Ca and Mg. Alterations of s-Mg^{++} within the normal range do not appear to affect the concentration of s-Ca^{++}. But hypomagnesemia tends to suppress PTH secretion and may lead to mild hypocalcemia. Changes in s-Mg^{++} level often reflect changes in s-Ca^{++}. low levels of Mg may be found in malabsorption and chronic kidney disease.

Table: The measured enzymes and biochemical parameters in normal control and pancreatitis patients

Parameters	Control (n=10) (mean±SD)	patients (n=20) (mean±SD)
S. Sodium (mmol/L)	145.8 ± 7.29	100.85 ± 10.11*
Calcium (mmol/L)	2.28±0.25	1.17±0.31*
Albumin (g/L)	43.0±6.12	26.85±4.08 *
S.amylase (U/L)	63.4±10.84	259.8±54.04*
TG (mmol/L)	1.5±0.52	4.09±0.78*
Potassium (mmol/L)	4.48±0.83	2.3±0.41*
Glucose (mmol/L)	5.6±0.95	9.34±1.06*
S.urea (mmol/L)	4.2±1.06	9.63±1.44*
Total bilirubin (mmol/L)	9.0±1.36	21.68±3.96*
Magnesium (Mg/ml)	21.6±1.33	11.035±2.51*
LDH (U/L)	126.8±54.51	263.65±38.57*
S. AST (S. GOT) (U/L)	15.2±3.96	37.8±9.53*

* significant differences at $p \leq 0.05$
n= number of samples.

References:

1) Goodman C. C. and Fuller K. S., "Pathology: Implications for the physical therapist". 3rd Ed. Saint Louis, MO, Saunders, pp109-118 (2009).

2) Beers M. H., "The Merck Manual of diagnosis and therapy". 18th ed. White House Station. NJ. Merck Research Laboratories, pp243, 272 (2006).

3) Lankisch P. G., Burchard R. S. and Lehnick D., Gut., 44:542-544 (1999).

4) Byrne M. F., Mitchell R. M., Stiffler H., Lemli M., Edwar J., Can. J. Gastroenterol, 16: 849-854 (2002).

5) Al-Bahrani A. Z. and Ammori B. J., Clinica Chimica Acta., 362:26-48 (2005).

6) Foitzik T., Klar E., Buhr H. J. and Herfarth C., Eur. J. Surg., 161:187-192 (1995).

7) Buter A., Imrie C. W., Carter C. R. et al., Br. J. Surg., 89:298-302 (2002).
8) Johnson C. D., Pancreas, 25:435 (2002).
9) Steinberg W. and Tenner S., New England J. of Med., 330 (17), 1199 (1994).
10) National Digestive Disease Information clearing house (NDDIC). Pancreatitis. http:// www. digestive. niddk. nih. gov/ ddiseases/ pub/ pancreatitis / (accessed2/March2010).
11) Nayar M., Charnely R., Scott J., Haugk B. and Oppong K., J. of pancreatitis, 10(5): 539-542 (2009).
12) Mast J., Morak M., Brett B. and Eijck C., J. of pancreas., 10(1): 53-54 (2009).
13) The National pancreas foundation.Volken P.V. http://www. pancreas foundation. org/learn/ pancreatitis.Shtml / (accessed21 /March2010).
14) Branks P., Am. J. Gastroenterology, 92(3):377-386 (1997).
15) Magill P., Ridgway P. Conlon K. and Neary P., J. of the pancreas, 7(3): 311-314 (2006).
16) Ran B., Cebulla M., UhI W., Schoenberg M. H. and Beger H. G., pancreas, 17:134-139 (1998).
17) Ambrose M., Nursing, 96:33-39 (1996).
18) Legaz M. E. and Kenny M. A., Clin. Chem., 22:57-62 (1976).
19) Dean J. A. "Flame Photometry". Mc GRAW-HILL Book Company, ING. New Yourk. Tornto. London, 3, (1960).
20) Reitman S. and Frankel S., Am. J. Clin. Path., 28:56 (1957).
21) Corns C. and Ludma C., Ann. Clin. Biochem., 24:345 (1987).
22) Tietz N. W., "Text book of clinical chemistry". 2nd ed. Saunders Company, U.S.A.: 13211-6 (1994).
23) Fossati P. and Prencipe L., Clin. Chem., 28(1):2077 (1982).
24) Doumas B. T., Waston W. A. and Bigg H. G., Clin. Chem. Acta., 31:87-96 (1971).
25) Toro G. and Ackermann P. G. "Practical Clinical Chemistry". Little, Brown and Company (Inc.), U.S.A.:497-506 (1975).
26) Trinder P., Ann. Clin. Biochem., 6:24-27 (1969).
27) Boehriger M., Scand. J. Clin. Lab. Invest., 33:291-306 (1974).
28) Matull W. R., Perira S. P. and O`Donhue J. W., J.Clin. Pathol., 59(4): 340-344 (2006).
29) Steiner J. M., Vet. Clin. North. Am. Small Anim. Pract., 33(5):1181-1195 (2003).
30) Smotkin J. and Tenner S., J. Clin. Gastroenterol, 34:459 (2002).
31) Branks P. and Freeman M., Am. J. Gastroenterol, 101:2379-2400 (2006).
32) Durrington P. N. and Miller J. P. Br. Medical J., 292, 15 (1986).

33) Lesser P. B. and Warshaw Al: Diagnosis of pancretitis masked by hyperlipemia. Ann. Inter. Med., 82(6):795-798 (1975).
34) Durrington P. N. & Miller J. P., Br. J. Hosp. Med., 32:28-34 (1984).
35) Russo M. W., Wei J. T. Thiny M. T and et al., Gastroenterology, 126: 1448-1453 (2004).
36) The patient education institute, Inc. www.x-plain.com (2009). http:// www.nlm.nih.gov/med.
37) Pancreatic abscess 2010 available at http://www.emedicine.medscape. com/article/181264-over view.
38) Parodi H. C., Gutierrez. S., Lattanzi M. and Martinez R., Acta. Gastroenterol Latinoam, 20(3): 137-144 (2000).
39) Mikael P., Joaquin J. E. and Sirius C., Gastroenterol. Res. Pract., 2009 (10): 1155 (2009).
40) Guyton A and John H. J., "Text book of Medical physiology", Saunders, (2005) ISBN 978-0-7216-0240-0.
41) Kazmier S. C., Carton S. C and Van. P. G., Clin. Chem., 41(4): 523-531 (1995).
42) Mayer A. D. and Mc Mahon M. J., Ann. Surg., 201 (1):68-75 (1985).
43) Mc Mahon M. J., Pickford I. R., Lancet, 2:541-543 (1979).
44) Davidson S. R., Neoptolemos J. P., Lesse T. and Carr-Locke D. L., Br. J. Surg., 75:213-5 (1988).
45) Grau F., Almela P. and Aparisi L., Int. J. Pancreatol., 25:107-111 (1999).
46) Bechieu U. WU, Richard S. J., Xiaowa S., Darwin L. C. and Peter A. B., Gastroenterology, 137:129-135 (2009).
47) Hess R. S., Saunders H. M., Vanwinkle T. J., Shofer F. S. and Washabau R. J., J. Am Vet. Med. Assoc., 213:665-670 (1998).
48) Ruaux C. G., and Atwell R. B., Aust. Vet. J., 76:804-808 (1998).
49) Son P., J. Vet. Sci., 1(1):6-65 (2000).
50) Senior D. F., "Text book of veterinary internal medicine: diseases of the dog and cat", 3rd ed., WB Saunders, Philadelphia, pp. 429-449 (1989).
51) Schaer M., Vet. Clin. North. Am. Small Anim. Pract., 12:399-409.
52) Matul W. R., Pereira S. P. and O`Donhue J. W., J. Clin. Patho., 59:340-344 (2006).
53) Goldstein D. A., International J. Gastrointestinal Cancer, 6(4): 249-262 (1990).
54) Kimmel S. E., Washabau R. J. and Drobat K. J., J. Am. Vet. Med. Assoc., 219(8):1105-1109 (2001).