MORPHOLOGY OF BLOOD SERUM IN ACUTE PANCREATITIS

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Abstract

Introduction

The problem of early diagnostics and prognosis of severe acute pancreatitis (AP) has not solved yet. Multifactorial scoring systems are good predictors of pancreatic necrosis and its severity. They have similar accuracy, but consume 48 hours for the full assessment. Inflammatory markers are not widely available. The distinction between interstitial and necrotizing pancreatitis by contrast enhanced CT scan can be reliably made after 2–3 days of hospitalization. The study was performed to clarify the morphological changes in blood serum in different course of AP and how it can help in its prognosis.

Patients and methods

There were examined 51 patients with AP treated in Hospital №35 in Nizhny Novgorod in 2009–2010. They were grouped according to Atlanta classification. Group 1 (n=24) – cases of necrotizing pancreatitis, group 2 (n=37) – interstitial pancreatitis. Blood samples were taken on admission, 12 h after admission, and every 24 h within first 3 days and further in different periods in the course of the disease. Serum specimens were dried in standard conditions and then examined by means of light microscopy.

Results

There was found a meshwork in the peripheral zone of dried serum specimens in all cases of severe AP with lethal outcome (n=4). Crystal structures in form of dendrites were observed in central area in 16 patients with necrotizing pancreatitis and in 2 – with interstitial form (p=0.0003). The hyperpigmentation in the boundary zone was seen in all cases. There was a weak correlation between severity of AP and intensity of hyperpigmentation (r=0.31; p=0.024). The mentioned phenomenon was particularly marked in biliary pancreatitis. Arnold’s structures were in the peripheral zone in both groups, but only in 3 patients with severe AP and multiple organ failure.

Conclusions

AP causes marked changes in biochemical status of the body that are reflected in morphological picture of blood serum. The presence of crystal structures in central area of dried serum specimen is indicative of high probability of necrotizing pancreatitis. A meshwork in the peripheral zone is associated with a severe AP and poor prognosis. Arnold’s structures are a positive sign of localization of the inflammatory process.

Keywords

Acute pancreatitis, Prognosis, Early diagnostics, Morphology of blood serum
be correctly evaluated by contrast-enhanced CT and ultrasonography in these periods. On the other hand, multiple organ failure develops in approximately 10% of patients with IP [15]. At present, the problem of early diagnostics of severe AP is not considered to be solved. The morphology of blood serum in AP is of great interest when estimating pathological changes in homeostasis in their unity. Recently the notion “morphology” in biology has been referred only to cell tissues while biological fluids have not been included into morphology research. In the study there was used a method of wedge-shape dehydration developed by Shabalin V.N., Shatokhina S.N. [16], which makes it possible to analyze morphological structure of blood serum. Using the mentioned method of dehydration of a drop of blood serum, a very thin film (facia) is obtained that is actually a fixed “section” of the studied fluid. The process of dehydration has a phase character because of unequal drop thickness in the center and periphery, the difference in osmotic and oncotic forces. The facia structure presents an integrated figure of all existing in biological fluid complex molecular interconnections that are regulated and transformed onto macroscopic level in a special way. The facia of blood serum in physiological status of organism is characterized by zonality, symmetric localization of radial fissures, right-angled and round elements. The objectives of the study were to clarify the morphological changes in blood serum indifferent course of AP and how it can help in its prognosis.

PATIENTS AND METHODS

51 patients with AP were treated in Hospital №35 in Nizhny Novgorod in 2009–2010. They were grouped according to Atlanta classification. Group 1 (n=24) – PN cases, group 2 (n=37) – IP. There were 25 patients with severe AP and 26 – with a mild form of the disease. Pancreatic necrosis was verified by ultrasonography, contrast-enhanced CT, magnetic resonance imaging, histological analysis and intraoperatively. Blood samples were taken on admission, 12 h after admission, in 24 h intervals within first 3 days and further in different periods in the course of the disease. A drop of blood serum (V=20 μl) was dried on the slide at 20°C, relative humidity 65% and minimal air flow. The duration of drying was 3 h. The investigation of structural elements of blood serum was made by means of light microscopy. The comparison of two independent groups was carried out using Mann-Whitney U test. Spearmen rank was determined for the purpose of correlation analysis.

RESULTS

The investigation of blood serum morphology revealed a number of phenomena, occurrence and intensity of which differed in mild and severe AP. There was found a meshwork in the peripheral zone of dried serum specimens in all cases of severe AP with lethal outcome (n=4) (Fig. 1). The sign was seen on the first day of the disease and remained till the death of the patient in spite of multi-component intravenous medication. Similar changes in the peripheral zone were observed in other two patients with NP who survived, but the course of the disease was accompanied by MODS, pancreatic abscesses, fistulas formation. The development of complications was the cause of reoperations and a prolonged stay in an intensive care unit. The finding of the meshwork in survived patients was non-persistent. Crystal structures in the form of dendrites branching at an angle 60–90° were observed in central area in 16 patients with PN both with and without MODS and in 2 – with interstitial form (Fig. 2). The groups differed significantly regarding the presence of the sign (p=0.0003) (Fig. 3, 4).

The evolution of appearance or disappearance of such crystals reflected different periods in the course of AP: clinical improvement, MODS reduction, the decrease of intoxication or complications development. The sign was continuously revealed in blood specimens in the cases with lethal outcome (Fig. 5, 6). The hyperpigmentation in the boundary zone was seen in all patients (Fig. 7). There was a weak correlation between severity of AP and intensity of hyperpigmentation (r=0.31; p=0.024). The mentioned phenomenon was particularly marked in biliary pancreatitis and chronic alcoholism. An infusion therapy led to the disappearance of hyperpigmentation within 1-2 days or the decrease in its intensity (Fig. 7, 8). In PN the present sign persisted during 5–10 days, in several cases – longer time. Crescent formations were seen in the peripheral zone in 13 patients, and only in 3 of them with PN (Fig. 9). These formations were difficult to identify in the presence of meshwork, haemolysis. Thus, they could not be estimated in quite a number of patients. Arnold’s structures were in the peripheral zone in 23 persons: 11 of group 1 and 12 of group 2, but only in 3 patients with severe AP and MODS (Fig. 10). Arnold’s structures were more expressed in cases of pancreatic infiltrate.

DISCUSSION

Pathogenesis of AP presents a cascading process, which is poorly understood especially on the molecular level. Inappropriate intracellular activation of proteolytic enzymes and their inflow in interstitium causes cytokines reactions [17]. The substances produced by polymorphonuclear leucocytes and other cells under the influence of cytokines damage phospholipid membranes not only in pancreatic tissue, but in
different organs [18, 19]. Owing to this, the products of cells degradation, inflammatory mediators, lipid peroxides come in blood. All these factors together with hypoxia, acidosis and energy deficiency lead to deep disturbances in homeostasis [20]. The function of blood proteins is altered, that is reflected in serum's structure formation. Particularly the coupling capacity of albumins decreases to a considerable extent [21, 22, 23]. Albumins were shown to localize in the peripheral zone by wedge-shape dehydration of blood serum [24]. As a result of albumins conformation, changes in the number and form of fissures of dried serum specimen occur [25]. The meshwork observed in patients with extremely severe course of AP and lethal outcome can be an evidence of significant abnormalities in the structure and function of albumins due to endotoxemia. The detection of the sign mentioned is considered to be prognostically unfavorable. The other aspect of changes in molecular interactions in blood serum is crystal structures formation in central area. A crystal form is known to be determined much by the elements of environment, i.e. by other dissolved organic and mineral substances. A type of crystallization correlates with the severity of pathology [16]. Basic crystal types could be seen in healthy subjects by means of polarization microscopy or in dark background with
lens magnification ×100. Crystals revealed were large (up to 0.5 mm), clear distinguishable even with lens magnification ×25 or less. We suggest the formation of such structures to be due to the imbalance between saline and organic components of serum, especially marked in severe PN. In both groups there was seen hyperpigmentation in the boundary zone, where globulin fraction localizes. The majority of acute phase proteins belong to globulins, the synthesis of which is carried out in the liver. In the course of the research there was not found a strong correlation between hyperpigmentation intensity and AP severity. However, acute phase proteins concentration does not always reflect PN severity [3]. On the other side, liver function alters more and earlier in individuals with chronic alcohol abuse and biliary etiology of AP that can influence the hyperpigmentation appearance. Crescent formations were found in 13 patients from 51 and in view of the fact, that it could be difficult to identify in quite a few cases, its diagnostic significance was not determined in our investigation. Arnold’s structures were described in acute and chronic inflammatory processes including
The latter fact is confirmed by analysis of blood serum in different periods of the disease during the treatment.

**Conclusion**

Deep disturbances in homeostasis in AP are reflected in morphological picture of blood serum. Revealed markers have no specificity relative to AP, but characterize the degree of pathological changes. The presence of crystal structures in central area of dried serum specimen indicates a high probability of PN. A meshwork in the peripheral zone is associated with severe AP and poor prognosis. The maintenance of mentioned signs in series of tests is the most unfavorable. Arnold’s structures are positive signs of localization of the inflammatory process. Thus, the investigation of blood serum by means of wedge-shape dehydration makes it possible to determine the severity of AP and predict the outcome in some cases. The method is available in any hospital.

**References**