Міністерство охорони здоров'я України
Вищий державний навчальний заклад України
«Буковинський державний медичний університет»

БУКОВИНСЬКИЙ МЕДИЧНИЙ ВІСНИК

Український науково-практичний журнал

Заснований у лютому 1997 року
Видається 4 рази на рік

Включений до Ulrichsweb™ Global Serials Directory, наукометричних і спеціалізованих баз даних Google Scholar (США), Index Copernicus International (Польща), Scientific Indexing Services (США), Infobase Index (Індія), НБУ ім. Вернадського, "Джерело" та до бази даних Всеросійського інституту наукової і технічної інформації Російської академії наук

ТОМ 21, № 1 (81)

2017

Чернівці: БДМУ, 2017

Рекомендовано до друку та до поширення через мережу Інтернет рішенням вченої ради Вищого державного навчального закладу України «Буковинський державний медичний університет» (протокол № 8 від 23 лютого 2017 року)

Буковинський медичний вісник (Бук. мед. вісник) – науково-практичний журнал, що рецензується
Bukovinian Medical Herald (Buk. Med. Herald)

Заснований у лютому 1997 р.
Видається 4 рази на рік

Founded in February, 1997
Published four times annually

Мова видання: українська, російська, англійська

Сфера розповсюдження загальнодержавна, зарубіжна

Свідоцтво про державну реєстрацію:
серія КВ №15684-4156 ПР
від 21.09.2009

Наказом
Міністерства освіти і науки України
від 06 листопада 2014 року № 1279
журнал
“Буковинський медичний вісник”
включено до
Переліку наукових фахових
видань України

Адреса редакції: 58002, Чернівці,
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УДК 616.37-002.2-018.2-008.93-056.527

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CONDITION COMPONENT OF CONNECTIVE TISSUE AND SYSTEMIC PROTEOLYSIS IN PATIENTS WITH CHRONIC PANCREATITIS AND OBESITY

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Abstract. 50 patients with chronic pancreatitis (CP) aged from 45 to 63 including 23 patients with CP and normal body index (1st group), 27 patients with CP and obesity of the 1st degree (2nd group) were examined in the research. The diagnosis of CP exacerbation was made on the basis of anamnesis, clinical and ultrasonography findings with biochemical substantiation of hyper-fermentation syndrome. The diagnosis of obesity was made on the basis of the body mass index increasing (BMI) more than 30 kg/m^2. A comparative analysis of the morphological structure of the pancreatic tissue received during autopsy of patients with CP on the background of obesity and normal body mass, died due to various reasons including complications of metabolic syndrome, has been made. In obese patients pancreas morphologically characterized by replacement of the pancreas acinar epithelial by fibrous tissue and a significant increase the number of adipocytes, which is the basis of exocrine pancreatic insufficiency.

Patients with chronic pancreatitis and obesity presented considerable metabolic changes of the extracellular matrix components assuming a reliable increase of the intensity of collagen synthesis, glycoproteins content, molecular hyper-production of cellular adhesion and acute phase proteins, increased catabolism of fucoglycoproteins against a reliable decrease of seromucoids and ceruloplasmin content with antioxidant properties in the blood. Increase of proteolytic blood activity and inhibition of collagenolysis processes due to the decrease of matrix metalloproteinase-1 activity, increase of inhibitory effect power of the tissue and plasma inhibitors of proteolysis and collagenolysis were found in patients with CP on the background of obesity.

Key words: chronic pancreatitis, obesity, autopsy, pancreas, proteinase-inhibitory imbalance, connective tissue, metabolism.

Progressing of chronic pancreatitis (CP) of any etiology is based on proteinase-inhibitory imbalance followed by diffuse fibrosis of the pancreas caused by the activation of the connective tissue system due to the destruction of the acinar epithelium, polymorphocellular infiltration of the pancreatic tissue with immune-competent cells under the influence of an increasing expression and activation of cellular adhesion factors, hyperproduction of pro-inflammatory cytokines, growth factors of anabolic action, acidosis, hypoxia etc. [1, 2, 4, 5]. A multi component system of collagen anabolism and carbohydrate-protein components of the extracellular matrix (ECM) is opposed by a powerful system of matric proteinases providing resorption of the scarred connective tissue for excessive amount [3].

At the same time, the system of neutrophilic granulocytes plays a considerable role in the development and progressing of CP, which factors of aggression are respiratory explosion with generation of active forms of oxygen and nitrogen, intensification of oxidative and nitrous stress as well as intraductal and tissue pancreas proteinase activation, active towards damaged protein substrates with focal self-digestion of the organ mainly [5, 6, 7]. The intensity of proteolytic processes is controlled by a number of tissue and plasma proteinase inhibitors (α_2-macroglobulin (α_2-MG), α_1-inhibitor of proteinase (α_1-IP), antithrombin III, tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) etc.) [3, 6, 8]. Imbalance of this system can result in prevailing of the processes of protein catabolism performing structural (cellular membrane components) and transport functions, which is a powerful damaging factor [8, 9].

The analysis of literary data is indicative of an inadequate investigation of the mechanisms of probable interrelations between the parameters of ana- and catabolism elements of ECM and proteinase-inhibitor system in pathogenesis of chronic pancreatitis progressing in patients with obesity.

Objective of the study: to find the availability of interrelations between the condition of functioning of the proteinase-inhibitor system and connective tissue in the mechanisms of progressing of the chronic pancreatitis in patients with obesity.

Materials and methods. 50 patients with chronic pancreatitis (CP) aged from 45 to 63 including 23 patients with CP and normal body index (1st group), 27 patients with CP and obesity of the 1st degree (2nd group) were examined in the research. The diagnosis of CP exacerbation was made on the basis of anamnesis, clinical and ultrasonography findings with biochemical substantiation of hyper-fermentation syndrome [2]. The diagnosis of obesity was made on the basis of the analysis of the body mass index (BMI) more than 30 kg/m2. All the patients of the 2nd group had obesity of the I degree. An essential condition to involve patients into the study was the absence of alcoholic and non-alcoholic fatty liver disease, systemic diseases of the connective tissue, rheumatic diseases, and other inflammatory diseases. In the 1st group of patients BMI was 23,2±1,8 kg/m^2, in the 2nd group of patients – 32,1±1,3 kg/m^2. The control group included practically healthy individuals (PHI) of an appropriate age and sex.

Metabolic changes of the extracellular matrix components were detected by the content off free oxyproline (FOP) according to S.S. Tetianets (1985) and protein-bound oxyproline (PBOP) according to M.S. Osadchuk (1979), hexosamines according to O.G. Arkhipova (1988), seromucoids (SM), sialic

acids (SA), fucose not bound with protein by means of the sets produced by "Danysh Ltd" (Lviv), ceruloplasmin by means of M.R. Revin's method (1976) in the blood. The content of matrix metalloproteinase-1 (MMP-1) and TIMP-1 in the blood was detected by means of immune-enzyme analysis (DRG System). The condition of the blood plasma proteolytic activity was studied by the total activity of the blood serum proteinases according to M. Kunits (1975). The intensity of low molecular proteinolysis (with azoalbumin), high molecular proteins (with azocasein) and collagen lysis (with azocol) was detected by means of reagents produced by "Danysh Ltd" (Lviv). The condition of proteinase-inhibitor system was studied by the content of α_2-MG in the blood serum, the content of α_2-IP in the blood plasma ("Danysh Ltd", Lviv). The results of the study were statistically processed by means of parametric and non-parametric methods of variation statistics.

Results of the study and their discussion. Considering the fact that chronic pancreatitis is chronic inflammatory disease of the pancreas characterized by irreversible destruction of the exocrine parenchyma and fibrosis, which results in destruction of the endocrine tissue of the gland at the late stages. At the preliminary stage of our studies [6] involving a comparative analysis of the morphological structure of the pancreas obtained during autopsy of patients with chronic pancreatitis against obesity and normal body weight died due to different causes including complications of metabolic syndrome, we have proved that in patients with obesity chronic pancreatitis from morphological point of view is characterized by statistically more valuable progressing fibrous reconstruction of the organ. Specifically, as far as fibrosing of the pancreas occurs, more pronounced dilation of the duct system, intensified curvature and variability of duct caliber are found against obesity. The gland stroma with obesity was considerably gross at the expense of newly formed collagen fibrils. Epithelium in the dilated ducts was thickened with the development of focal papillary hyperplasia and squamosal epithelial metaplasia in the ducts. Formed protein-mucous crusts, precipitates are more frequently found in dilated ducts mainly in the smallest branches of the common pancreatic duct. At the early stages of chronic pancreatitis proliferation of the connective tissue around the ducts and between the lobes, minimal or moderately pronounced inflammation, prevailing focal infiltration with T-lymphocytes were found in the parenchyma of the pancreas in patients with obesity. With progressing of the disease the connective tissue proliferates between acinuses that were deformed and absent in some places. In case of severe chronic pancreatitis the connective tissue replaced acinar structures completely, and the gland was mainly decreased in its volume and compact. The analysis of specimens of the pancreas obtained during autopsy of patients with chronic pancreatitis with normal body weight showed that islets of Langerhans remain even at the late stage of the disease while in case of chronic pancreatitis with obesity the islets were destructed in 50% and replaced by the connective tissue [6, 7].

Considering the fact that intravital biopsy of the pancreas in patients with obesity is a labour-intensive process and it can decrease the quality of life of those patients, we have analyzed the intensity of fibrous-forming reactions in the body of patients with chronic pancreatitis by the content of the connective tissue components, intensity of collagenolysis and plasma proteolysis in the blood. The investigations performed are indicative of the fact that patients with chronic pancreatitis have a considerable activation of fibrous-forming reactions in the pancreas: reliable increase of PBOP in the blood – a marker of collagen anabolism in both groups of comparison – in the 1st group of patients it was (75,62±3,43) mcmol/L, the 2nd group – (83,95±6,38) mcmol/L against (41,48±3,72) mcmol/L in the control group respectively ($p<0,05$). The tendency of changes of FOP content, a marker of collagen catabolism in patients of the 1st and 2nd groups differed ($p<0,05$). In particular, FOP content in patients with normal body weight became reliably 23,5% higher ($p<0,05$), and in patients with chronic pancreatitis and obesity it became 28,9% lower ($p<0,05$). The latter fact is indicative of that resorption of a newly formed connective tissue in patients with obesity is considerably reduced, and fibrous-forming reactions do not have appropriate resistance. The 2nd group of patients was also characterized by a reliable decrease of MMP-1 ($p<0,05$), which is caused by an increased content of TIMP-1 in the blood ($p<0,05$), and the changes of these indices in patients from the 1st group were unreliable (Table 1). Are liable decrease of the ratio between MMP-1/TIMP-1 in patients with chronic pancreatitis of the 2nd group is indicative of a considerable imbalance in the system of functioning of the connective tissue ($p<0,05$). Decreased intensity of collagenolysis in this group of patients is likely to promote the development of diffuse fibrosis of the pancreas tissue.

Are liable increase of the content of hexosamines and sialic acids, and decrease of the content of seromucoids and ceruloplasminin in the blood of patients with chronic pancreatitis of both groups of comparison ($p<0,05$) promote organization or "cementation" of collagen fibrils in the extracellular matrix and decrease probability of their resorption. Increase of fucose not bound with protein in patients of the 1st and 2nd groups ($p<0,05$) is indicative of the induction of fucoglycoproteins catabolism processes of the intracellular matrix and it is one of the markers of degradation of normal extracellular matrix [3].

In addition to the factors promoting the progress of pancreas fibrosis with chronic pancreatitis under conditions of obesity (activation of lipid peroxide oxidation, oxidative modification of proteins, the processes of acinar epithelium apoptosis, endotoxicosis, hyper production of cytokines:TNF-α, IL-1β and growth factors: TGF-β1, IGF-1, disorders of arterial perfusion of the pancreas) [2, 3, 5, 6, 7] there

Table

Indexes of proteinase inhibitory system in patients with chronic pancreatitis with normal body weight and obesity (M±m)

Indexes	PHI, n=30	CP (1st group), n=23	CP and obesity (2nd group), n=27
Lysis AA, E440/ml*hr	2,41±0,02	3,71±0,10 *	3,97±0,05 */**
Lysis AK, E440/ ml*hr	2,16±0,01	3,48±0,07 *	3,86±0,13 */**
Azocololysis, E440/ ml*hr	0,84±0,02	0,99±0,004 *	0,68±0,005 */**
MMP-1, мmg/l	8,17±0,84	9,11±1,28	4,51±0,17 */**
TIMP - 1, мcg/l	196,46±11,25	192,46±9,75	298,82±9,18 */**
Proteinaise, мcg/l	0,42±0,003	0,71±0,004 *	0,85±0,01 */**
α_2-MG mmol/ l	2,31±0,14	5,01±0,12 *	6,67±0,18 */**
α_1-IP, mkmol/ l	38,55±2,23	152,73±2,83 *	168,52±4,14 */**

Notes. * – Probable difference compared to the index in healthy individuals (P<0,05); ** – Probable difference compared with the rate in patients with CP (P<0,05)

are functional disorders of the proteinase-inhibitor system (p<0,05). This, in both groups of patients with chronic pancreatitis increased total activity of proteinases (p<0,05), intensity of high and low molecular proteins lysis were found (p<0,05) (Table 1). At the same time, more considerable increase of intensity of unlimited proteolysis was found in the 2nd group of patients than in the 1st group of patients (p<0,05). Attention was drawn to different tendencies of collagenolytic activity (CLA) changes in the blood plasma: in patients of the 1st group azocol lysis increased concerning the control (p<0,05), while in the 2nd group of patients it decreased (p<0,05) (Table 1). The changes found were accompanied by are liable decrease of MMP-1 (collagenase) in the blood of patients with chronic pancreatitis of the 2nd group of observation (p<0,05) as compared to the parameters in the control group. Binary correlation-regressive analysis conducted is indicative of available strong direct correlation link between the content of MMP-1 and CLA in the blood (r=0,72; p<0,05), between the content of FOP and CLA (r=0,61; p<0,05), which is indicative of dense interrelations of these processes.

The changes indicated are likely to occur due to considerable imbalance in the system of tissue and plasma proteinase inhibitors. In particular, we have found a considerable increase of α_2-MG and α_1-IP in the blood of patients with chronic pancreatitis (p<0,05) as compared to the control parameters according to the increase of body weight (Table 1). An imbalanced increase of proteolytic intensity, even under conditions of compensatory increase of their inhibitors activity in patients with chronic pancreatitis results in progressing destruction of the cellular membranes of the pancreatic cells, acceleration of their apoptosis and development of necrosis, aggressive degradation of the key components of the extracellular matrix of the pancreatic tissue [3, 4, 9]. Afore-mentioned factors are active inductors of both inflammation (with formation of cytolysis of the acinar epithelium) and fibrogenesis processes, that is pathologic reparation of the pancreatic tissue with the elements of the connective tissue with gross disorders of the in tissue architectonics and development of external secretory dysfunction of the pancreas [3]. Increase of CLA in patients with chronic pancreatitis and normal body weight enabled to balance the processes of ana- and catabolism of collagen in this group of patients. Induction of generation processes of proteolytic inhibitors with active forms of oxygen in case of chronic pancreatitis of patients with normal body weight resulted in less intensive proteolytic damage of cells. Patients with obesity presented maximal by the intensity increase of proteolytic processes and decrease of collagenolytic activity of blood plasma and MMP-1 content – a key enzyme of collagen degradation, resulting in diffuse fibrous formation of the pancreas with development of its external secretory failure.

Conclusions

1. Considerable metabolic changes of the components of extracellular matrix as suming are liable increase of intensive synthesis of collagen, glycoproteins, hyperproduction of cellular adhesion molecules and acute phase proteins, intensification of catabolism of fucoglycoproteins against reliable decrease of seromucoids and ceruloplasmin with antioxidative properties in the blood are found in patients with chronic pancreatitis in the background of obesity.

2. Peculiarities of imbalance between blood proteolytic activity and the content of proteolytic inhibitors in patients with chronic pancreatitis with normal body weight are increased intensity of proteolysis of low- and high-molecular proteins, collagenolytic blood activity against considerable compensatory increase of activity of the tissue and plasma proteinase inhibitors. Chronic pancreatitis with obesity promotes more intensive increase of blood proteolytic activity and inhibition of collagenolytic processes due to reduced activity of matrix metalloproteinase-1, increased power of inhibitory influence of the tissue and plasma inhibitors of prote-

olysis and collagenolysis. Binary and total correlation-regressive interdependence found between the indices of the condition of the connective tissue and proteinase-inhibitor system is indicative of interdependence of the changes mentioned and their role in pathogenesis of chronic pancreatitis progress.

Prospects of further studies are to investigate component exchange of the carbohydrate-protein components of connective tissue, condition of the proteinase-inhibitor system in interrelation with the condition of factors of coagulation hemostasis, processes of lipid and carbohydrate metabolism in patients with chronic pancreatitis and obesity.

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СОСТОЯНИЕ КОМПОНЕНТОВ СОЕДИНИТЕЛЬНОЙ ТКАНИ И СИСТЕМНОГО ПРОТЕОЛИЗА У БОЛЬНЫХ ХРОНИЧЕСКИМ ПАНКРЕАТИТОМ И ОЖИРЕНИЕМ

О.С. Хухлина, В.С. Смандыч

Аннотация. Обследовано 50 пациентов с хроническим панкреатитом (ХП) в возрасте от 45 до 63, включая 23 пациентов с ХП и нормальным индексом массы тела (первая группа), 27 пациентов с ХП и ожирением 1-ой степени (вторая группа). Диагноз ХП в фазе обострения был установлен на основании анамнеза, клинических данных и результатом ультразвукового исследования поджелудочной железы с биохимическим обоснованием синдрома гипер-ферментемии. Диагноз ожирения установлен на основании повышения индекса массы тела (ИМТ) более 30 кг/м^2. Проведен сравнительный анализ морфологической структуры ткани поджелудочной железы, полученной при автопсии пациентов с ХП и ожирением и нормальной массы тела, умерших из-за различных причин, в том числе осложнений метаболического синдрома. У пациентов с ожирением морфологически поджелудочная железа характеризуется замещением ацинарного эпителия фиброзной тканью и существенным увеличением количества адипоцитов, что составляет морфологическую основу внешнесекреторной недостаточности. У пациентов с хроническим панкреатитом и ожирением установлены значительные изменения компонентов внеклеточного матрикса, которые предусматривают достоверное повышение интенсивности синтеза коллагена, гликопротеинов, гиперпроизводства факторов клеточной адгезии и белков острой фазы, увеличение катаболизма гликопротеина, с одновременным снижением серомукоида и содержания церулоплазмина с антиоксидантными свойствами в крови. У пациентов с ХП и ожирением были обнаружены повышение протеолитической активности крови и угнетение процессов коллагенолиза за счет снижения активности матриксной металлопротеиназы-1, повышения тормозящего действия тканевых ингибиторов протеолиза и коллагенолиза.

Ключевые слова: хронический панкреатит, ожирение, аутопсия, поджелудочна железа, протеиназо-ингибиторной дисбаланс, соединительная ткань, обмен веществ.

СТАН КОМПОНЕНТІВ СПОЛУЧНОЇ ТКАНИНИ ТА СИСТЕМНОГО ПРОТЕОЛІЗУ У ХВОРИХ НА ХРОНІЧНИЙ ПАНКРЕАТИТ ТА ОЖИРІННЯ

О.С. Хухліна, В.С. Смандич

Резюме. Обстежено 50 пацієнтів із хронічним панкреатитом (ХП) віком від 45 до 63, включаючи 23 пацієнтів із ХП і нормальним індексом маси тіла (1-ша група), 27 пацієнтів з ХП і ожирінням 1-го ступеня (2-га група). Діагноз ХП у фазі загострення був встановлений на підставі анамнезу, клінічних даних та результатів ультрасонографії підшлункової залози із біохімічним обґрунтуванням синдрому гіперферментемії. Діагноз ожиріння встановлено на підставі підвищення індексу маси тіла (ІМТ) більше 30 кг/м^2. Проведено порівняльний аналіз морфологічної структури тканини підшлункової залози, отриманої під час автопсії пацієнтів із ХП та ожирінням і нормальної маси тіла, які померли внаслідок різноманітних причин, у тому числі ускладнень метаболічного синдрому. У пацієнтів з ожирінням морфологічно підшлункова залоза характеризується заміщенням ацинарного епітелію фіброзною тканиною та істотним збільшенням кількості адипоцитів, що складає морфологічну основу зовнішньосекреторної недостатності.

У пацієнтів з хронічним панкреатитом і ожирінням встановлені значні метаболічні зміни компонентів позаклітинного матриксу, які передбачають достовірне підвищення інтенсивності синтезу колагену, глікопротеїнів, гіперпродукції факторів клітинної адгезії і білків гострої фази, збільшення катаболізму глікопротеїну, із одночасним зниженням серомукоїду і вмісту церулоплазміну з антиоксидантними властивостями в крові. У пацієнтів з ХП та ожирінням встановлено підвищення протеолітичної активності крові і пригнічення процесів колагенолізу за рахунок зменшення активності матриксної металопротеїнази-1, підвищення гальмуючої дії тканинних інгібіторів протеолізу та колагенолізу.

Ключові слова: хронічний панкреатит, ожиріння, автопсія, підшлункова залоза, протеїназо-інгібіторний дисбаланс, сполучна тканина, обмін речовин.

Вищий державний навчальний заклад України «Буковинський державний медичний університет» (Чернівці)

Рецензент – проф. О.І. Федів

Buk. Med. Herald. – 2017. – Vol. 21, № 1 (81). – P. 173-177

Надійшла до редакції 03.11.2016 року