Stress in cholecyst pathology

ILEANA VIORICA DINCA

Department of Human Anatomy, University of Medicine and Pharmacy of Craiova

ABSTRACT Chronic or acute daily stress, postoperative or food one represents a risk factor causing the appearance of the cholecystitis or acute or chronic cholecyst-pancreatic pathology. The present study focused on the extracellular matrix microanatomy from the cholecyst wall, correlated to the storing function of the gallbladder. “Neuromuscle units” revealed into the cholecyst wall structure, spacial distribution of the microanatomic elements from the vesicular wall and the relationships they have with the extracellular matrix structure determine that the cholecyst wall should react as a unitary whole in case of an excessive neurotransmission.

KEY WORDS neuromuscle units, cholecystic pathology, stress

Introduction

Cholecyst pathology was and it still represents a very common cause of morbidity all over the world, and that’s the reason for it can be considered the most expansive pathology of the digestive tract, therefore, explaining the interest in the liver and biliary ducts studies from the earliest times (1550 B.C.). The results of the studies in anatomy, physiology, biology separately taken, did not succeed in bringing conclusive answers for the clinicians, concerning both the initiation of a cholecyst pathologic process; and why the factors considered etiopathogenic ones (age, sex, lifestyle, food habits, etc.) can represent risk factors in that pathology production.

Having the same questions as a basis, we performed a study on a group of 200 patients diagnosed as having cholecystic pathology, study which took into account the cholecystic wall microanatomy, its elements stereodistribution, the relationship existing between the microanatomic and the structural elements of the extracellular matrix from the cholecyst wall and also their reaction way when the inflammatory process appeared.

Correlating all those microstructures to the functional relationship in interdeterminism which existed between the extracellular matrix and the cells afferent to it, into the cholecyst wall I tried to infer the mechanisms inducing the cholecyst pathology.

Materials and Methods

Cholecystectomy pieces from 200 patients operated for cholecyst pathology during the period of 2006 in the surgery Clinic II of the Emergency County Clinic Hospital of Craiova. Processing the pieces in classic staining was made within the Nucleus of Scientific Researches of the Laboratory of Biomedical Researches of the Medical Sciences Academy being placed into the “Filantropia” Hospital of Craiova; immunohistochemical study was made within the “Victor Babes” Institute of Bucharest.

As to reveal the structural elements of the gallbladder wall, we used classic stainings which were selected according to the investigator’s aim; hematoxylin-eosin staining to reveal the general histotopographic orientation of the structural elements; blue methylene to reveal the structural conjunctive; we used Van Gieson staining to reveal collagenic structures and Gomori silver impregnation to reveal the reticulinic fibres differentiation (collagen III); we also used Giemsa staining to emphasize mucopolysaccharids and glycosaminoglycans differentiation was showed off by means of Schiff Periodic Acid (PAS).

As to get details on the extracellular matrix and also on the way in which both the extracellular matrix components and cells reacted when the pathologic process appeared, we used techniques based on the antigen-antibody reaction (immunohistochemical): monoclonal antibody CD68 (KP1) was used as a marker for mastocyte-macrophage line; epithelial cells from the mucous tunic of the cholecyst was revealed by the monoclonal antibody CD34 made by DAKO; we used anti collagen IV and anti laminin antibody to reveal the basal membrane; the affected extracellular matrix afferent cells way of reaction was marked
by cytoskeletal stress antifibres antibody: anti actin and anti desmin antibodies.

**Results and Discussions**

On the pathologic cholecyst pieces examined by us, the cholecyst muscle layer appeared hypertrophied, with condensations of collagen fibres, the muscle fascicles presenting an alternance of their spacial orientation: longitudinal, oblique and circulary, giving the impression of a plexiform spacial disposition of the three muscle layers, classically described (figure nr.1B); they were closely joined by picrofuxinophyle collagen fibres following their trajec. Into the interfascicular spaces, we easily noted the presence of arterioles and perimyocitary meta-arterioles closely following the muscle fascicles trajec (figure nr.1 A,B); they were also sectioned in different planes.

**Figura nr. 1** Alternanţă de orientare spaţială a fasciculelor de celule musculare: circulară şi oblică. În spaţiile interfasciculare se remarcă cu uşurinţă prezenţa de metaarteriole şi capilare perimioicatere. Tunica externă conţine o stromă laxă bogată în fibre colagene picrifuxinofile cu traiect spiroidal. 1. Cripte; 2 Repliuri; 3. Fascicule de fibre musculare oblice şi longitudinale în tunica musculară; 4. Tunica seroasă. Coloraţie: Van Gieson (A;B); oc. 7; ob. 10 (A; B); x 70 (A; B). Cazul nr. 142 B. G. 70 ani; Colecistită cronică litiazică.

We also observed that the vessels muscle tunic had direct relationships to the perivasculary fibroconjunctive tissue and could also noted that the perivasculary fibroconjunctive tissue had continuity relationships to the vessel extramuscle vascular layer (figure nr.2A,B); it seemed that vascular adventitia was formed by that latter condensation and the vessel was anchored to the fibrocollagen elements of the perivasculary matrix, thus making the vessel lumen remain largely opened (figure nr.3A).

**Figura nr.2** Tromboze arteriale recanalizate în peretele vezicular (A); Tromboze venoase vechi recanalizate (B); Stromă perivasculară cu intensă angiogeneză. 1. Arteră trombozată; 2. Tromb arterial recanalizat; 3. Venă trombozată; 4. Tromb venos recanalizat; 5. Proces de angiogeneză perivascular. Coloraţie: hematoxilină-eosină (A; B); oc. 7; ob. 5 (A); ob. 10 (B); X 35 (A); X 70 (B). Cazul nr. 78 C. M. 67 ani; Colecistită cronică litiazică.

There existed the possibility that the three layers of smooth muscle fibres classically described (longitudinal, oblique, circulary) continuing to each other, should have a plexiform spacial stereodistribution and determine a general contraction to the cholecyst wall (similar to the myometrium and urinary bladder detrusor) in spite of a peristaltic one such as at the intestinal tube level. (In fact, we didn’t found in literature the description of the cholecystic wall contraction but, as it had appeared in the cholecystographic images, it suggested a global and concomitant contraction of the entire vesicular wall).

Under those circumstances it’s not surprising that the vessels closely following the muscle fibres trajec, by repeated contractions overwhelming the physiologic, microcirculation of the cholecyst wall was affected, with the initiation of an overrequirement process of the functionality in indeterminism of both the matrix and cells afferent
to it, with gradually hyperproduction of matricial elements, fibrosis and disfunctionality subsequently and gradually appeared into the cholecyst wall, followed by the gallbladder stasis and initiation of the vicious circle between the bile and the vesicular wall, thus determining the appearance of the pathologic process.

We could note that the vessels were closely joined by the nervous fillets which were likely to have perivascularly winding tract, into the muscle tunic. On the transversal sections, fascicles of nervous fillets presented a densification of collagen fibres around them (figure nr.3A,B) from which very fine collagen fibres started towards the nervous fascicle; they were likely to anchor the nervous fascicle entirely to the fibrocollagen densification around it.

![Figura nr. 3 Matrixul extracelular din lamina propria are raporturi cu epiteliul mucoasei format din celule cilindrice ciliate şi realizează axele conjunctive ale repliurilor mucoasei; vasele sanguine sunt înconjurate de fascicule de fibre colagene ce realizează punţi de ancorare a vaselor la tunica musculară. 1. Lamina propria; 2. Tunica musculară; 3. Venulă; 4. Arteriolă; 5. Nerv secţionat oblic; 6. Epiteliul mucoasei. Coloraţie: Van Gieson; oc. 7; ob. 20 (A); ob. 40 (B); x 140 (A); x 280 (B). Cazul nr. 20 D. L. 53 ani; Colecistită cronică parahidatică.](image)

The study of the extracell matrix elements classified those fine collagen fibres as being collagen IV belonging the collagen family FACIT (Prokop 1995; Ricard Bloom, 2000) having an anchoring role of the vasculo-nervous structures to the fibrillar collagen of the extracell matrix (figure nr.4 A,B). It was also noted that fascicles of conjunctive fibres around the nervous filament protruded into the nervous fascicle and continued with those crossing and compartmentalizing the nerve, at its periphery, the fine collagen IV fibres anchored to the collagen fascicles protruding the nervous fillet (figure nr.4 A,B).

![Figura nr. 4 La examinarea cu obiectivele 20x şi 40x, numeroase fibre colagene izolate (1) sau grupate (2) brâzdează şi compartimentează nervul pe o secţiune în plan transvers. La periferia nervului se remarcă prezenţa de fascicule de fibre colagene cu traiect sinusoidal (3)ce se continuă cu fibre colagene ce compartimentează nervul(4) şi fascicule colagene fine ce par să ancoreze nervul (5) . Coloraţie: Van Gieson; oc. 7; ob. 20 (A); ob. 40 (B); x 140 (A); x 280 (B). Cazul nr. 20 D. L. 53 ani; Colecistită cronică parahidatică.](image)

On the preparations upon which the section interested a nervous fillet transversally and then obliquely sectioned, we observed that bundles of fascicles of muscle fibres; the matrix around both the elements (nerve and muscle fascicles) were crossed by many meta-arterioles (figure nr.5A).
By using a 40x objective, we observed that miocyte fascicles around the nervous fillets intersected in variable planes, thus achieving a plexiform network with rhombooidal meshes crossed by fine meta-arterioles (figure nr.5B).

On a Gömöri silver impregnation we noted that collagen fibres crossing the miocyte fascicles had continuity relationships to the collagen fibres from the extracell matrix coterminous to the muscle fascicles and they achieved a plexiform network with small meshes where miocytes of the muscle fascicles and meta-arterioles were caught (Figure nr. 6).

With 20x and 40x objectives we observed that collagen fibres of plexiform network from the muscle fascicles achieved continuity relationships to the collagen fibres network around the nervous fascicle, and this one, in its turn, goes on with collagen fibres dissecting the nervous fascicles in different planes (figure nr. 7).
Figura nr. 7 Raporturile de continuitate și contiguitate între rețelele de fibre de reticulină ale componentelor neuronale și musculare ale „unităților neuromusculare” din tunica musculară a colecistului. Ele sunt înconjurate de un matrix extracelular lax. 

Colorație: Gömöri; oc. 7; ob. 20; x 140 Cazul nr. 20 D. L. 53 ani; Colecistită cronică parahidatică. 

By the carefully examination of the transversal and oblique and longitudinal sections of the nervous fascicle, we noted that the collagen fibres dissecting the nerve were sectioned both in transversal and oblique and longitudinal planes giving the tridimensional impression of a more lax plexiform distribution within the nervous plexus. As a consequence, we could say that the plexiform collagen network from the level of the muscle fascicles joining the nerve, was continuous to the more lax plexiform network from the inside of the nervous plexus and all the ensemble seemed to make up a “neuromuscle unit” surrounded and crossed by meta-arterioles and anchored to the matricial tissue fibres around. The presence of those “neuromuscle units” into the microanatomic structure of the cholecyst wall, could explain, by correlating them to the functionality relationship in interdeterminism of the extracellular matrix with the cells, by means of the basal membrane, another mechanism another vicious circle-vesicular bile initiation and more chronic or acute affection of the cholecyst releasing, thus advancing up to the neoplastic phenomenon. 

The fact that fibrillar elements of the extracellular matrix from the gallbladder wall was continuous to those from the muscle fascicles and continued to that around the nervous fillet neurofibrils; but us we could see the nervous fillet from the vascular wall, by studying the extracellular matrix stereodistribution and its interrelation to the cells by means of the basal lamina which interposed between the matrix fibrillar “skeleton” and cells, then, it gave us a very plastic spacial image and too simplistic perhaps, for such phenomena, the image of an extremely branchy tree (represented by the matrix), constituting the “skeleton” of the organ tissue respectively, communicating the “leaves” (represented by cells) by means of the basal lamina. It is known that basal lamina forms a continuous layer under epithelium and a muff around the cells including the nervous ones. 

(Yurchenko 1994; Dziadek 1995; Timple 1996; Kohorn 2000; Panos 2004). And then, the matricial environment with its fibrillar collagen around miocytes, would communicate to them by means of the perimyciary basal lamina which was also continuing along the fibrillar collagen and it would go on with the matrix within the nervous fascicle covering each neurofibrill to which it would communicate by means of the basal lamina surrounding it. It is known that, neurotransmission at the synaptic level is made by neurotransmitters. 

Unlike the skeletal muscles, the smooth muscle fibres had not neuromuscle plate, therefore, neurotransmission would be made by means of the basal lamina around each neurofibril (it is known that at a distance of 50-100µ of synapse neurofibril lost its myelin sheath), basal lamina, in turn, transmitted it by means of the membranary receptors of the muscle cells. Neurotransmitters present into the neurofibrills from the wall of the gallbladder were represented by those ubiquitarily found at the synapses level from all the organs: cholinergic, adrenergic, dopaminergic, etc., but also a cholecystokinina-neurotransmitter which-though it seemed to be secreted by all the neurons (Virgil Dinca, 2005; Radu Suciu, 2008) however it seemed to act specifically only at the level of the synapses from the gallbladder and pancreas walls, cholecystokinina neuronal synthesis was stimulated by the duodenal chyme lipoproteic enriched. It is also known that neurotransmitters synthesis started from tyrosin, triptofan, choline, histidine and each neurotransmitter had its specific postsynaptic receptor. 

Thus, postsynaptic transmembranary receptors would have tyrosinic, cysteinic, etc. situses in their structure which, when recognized by the
neurotransmitter, should activate the focal adhesion hotbed, from the level of which, it should be coupled to the actinic stress fibres and cytoskeletal desminic and, to the AP-1 nuclear transcription factor, by means of small G proteins (rac, raf, ras) and of the molecular adaptors (p-src, cdc2, erb). But, intracellular adaptation proteins coupled, at the same time, at the focal adhesion hotbed initiated by the receptor, but also to Zo-1 and Zo-2 proteins which intracellularly transmitted information about the intercellular adhesivity.

As a consequence, the cell would transmit response back to the surrounding matrix, but also to the intercellular adhesion proteins thus influencing the intercellular space increase or decrease at the level of the cholecyst mucosa epithelium, therefore, the resorption process paracellularly increase or decrease. The presence of the argentaffine materials in those intercellular spaces was a proof of the matricial cholinergic type elements also disposed into the intercellular spaces, as a component part of the desmosomal type intercellular adhesion “apparatus” or the presence of the transmembranary collagens having a role in adhesion and signalizer or/and a proof of the collagen excessively secreted or excreted by the hyperactive epithelial cells.

Those facts could explain numerous extremely complex ways in the matrix-cell functionality and also the mechanisms which pathology was induced through. Many experiments demonstrated that one of the causes inducing structural and microanatomic changes into the gallbladder wall and pathology, as a consequence, would be the microcirculating disorders; experimentally, microangiographs and echoplannary images in magnetic resonance (Gaudio 1993; Lim 1996; Hakala 1997; Ischiro Yamada 1999).

That is not to be disputed if we think about the vessels stereodistribution among the fascicles of miocytes if we take into account that the extracellular matrix elements from the structure of the gallbladder wall were continuous among them and they continued to those of the vessels and nerves walls; if we take into account that the last very fine ramifications of the vessels formed capillary plexus in the superficial subepithelial portion of the lamina propria, arteriolar capillars from that level were joined by a nervous plex and miocytes windingly or plexiformly disposed around the capillars (microcirculation proved by Osamu Othani’s electromicroscopic scanning in 1998), then it resulted that nervous impulses of an intensity and frequence overwhelming the physiologic, transmitted by means of the “neuromuscle units” of the vesicular wall and determining its contractility, would induce, on one hand, circulatory disorders and, on the other hand, changes of intercellular adhesivity with changes of resorption on a paracellulary way and, therefore, disorders into the matrix-cell functionality, with exceeding matricial elements cell secretion.

That’s how the motility disfunctions could be logically explained and, subsequently, the appearance of the cholecyst chronic affection as a starting point into the cholecyst wall, and having the “neuromuscle units” as a support and adrenergic, dopaminergic type neurotransmitters, from the time of the repeated and smaller intensity stress. By that mechanism could be explained the appearance of acute cholecystitis or acute cholecystopancreatitis of stress; as it is known that both cholecyst and pancreas have many neuroreceptors for those neurotransmitters.

Very strong stress, both the cotidian and postoperative ones, taking into account the stereodistribution of the microanatomic and matricial elements of the cholecystic wall, correlated to the functional interrelation between them, would determin, by the prolonged contracture of the vesicular wall, changes of vascularisation, matricial elements hyperproduction, resorption disorders at the level of the cholecyst mucosa; stress persistence and intensity didn’t allow recovering by matricial own means of the functional balance between matrix and cells.

Resorption disorders of mucosa epithelium, polysaccharidic and glycoproteic gels, rapidly and exceedingly produced, venous returning decreasing due to the prolonged muscle contraction into the cholecyst wall, all those would determine edema appearance with turgescence increase and cholecystic wall thickness given by the glycosaminoglycans exceedingly produced; edema, in turn, emphasized the vessels collabation thus achieving a vicious circle. All those had an echo upon the gallbladder; resorption changes at the epithelial levels and glycosaminoglycans secreted and exceedingly extravasated into the vesicular lumen, would change the concentration and the relationships among the gallbladder constitutive elements; the gallbladder acted, in turn, as an “irritative spina” upon the cholecyst wall, thus achieving a vicious circle. Structurally, necrobiosis with acute inflammatory infiltrate zones appeared (mastocytes and macrophages motility and migration were lightened by matricial glycosaminoglycans excessively produced); it also appeared neoformation vessels in full
inflammatory process (figure nr. 8 A) certifying tenascin glycoprotein presence [whose synthesis was proved to increase proportionally to both the inflammatory process and the fibroblasts mechanic aggression and their exposure to cytokinic interleukina-1 as it was proved to have an increased titre within angiogenesis (Heath 1989; Van Eyken 1992; Schuppan 1990; Erickson 1993; Lightener 1994; Hubbard 2000)].

Basal membranes were densified, doubled with collagen IV stratification, with subepithelial progenitor cells hyperactivity, with epithelial cells areas of apoptosis regenesis and even with epithelial cells desquamation from the basal lamina (figure nr. 8 B).

All those certified sudden hyperproduction of matricial elements but also that the matrix, by feedback methods, could not manage to “command” the cell secretion of matricial elements to decrease and that the metalloproteinases (MMPs) have been activated, more than that, the metalloproteinases and their inhibitors (MMPs/TIMPS) equilibrium was broken [as a proof that basal lamina was doubled, as it was known that the nidogene interconnecting the two polymeric protein networks from the basal lamina structure (lamina and collagen IV) presented in their structure a very sensitive field to proteases (Dziadek, 1995)]. Epithelial cells desquamation from the basal lamina also certified tenascins excessive presence which, among other features, presents another one such as: under certain (inflammatory) conditions, by their non-adhesive domains, they substituted fibronectine linked to syndecan-4 transmembranary proteoglycan (Wentao, 2001) thus decreasing the cell adhesion to matrix. As it was proved that the matricial elements synthesis was decreased by the corticoids [especially glycosaminoglycans of hyaluronic acid type (Schuppan, 1993; Ottensmeyer, 2000; Whitehead, 2000) then it might be explained how the corticosteroid administration reduced those phenomena making that the symptomatology in those patients should be improved.

By the same mechanism of neurotransmission, chronic, acute cholecystic or cholecysto-pancreatic affections could be released but, by means of cholecystokinin neurotransmitter, whose neurosecretion was stimulated by the lipoproteic enriched duodenal chyme as it is known that specific receptors for that neurotransmitter could be found both at the cholecystic wall cells level and into the pancreatic islands cells. Thinking about those exposed above and about the matrix-cells interrelation, we could state that persistent, repeated, more than necessary use of cholecystokinetic food might determine chronic cholecystic-pancreatic diseases in time, while, large amounts of that food used in a short time (especially after periods of religious abstinence) could lead to an acute cholecystic or cholecysto-pancreatic processes.

This mechanism would also explain the almost equal percentage resulted from the statistics upon the acute and chronic choledistis (15 and 15,7%) associated to acute or chronic pancreatitis. By the same action of cholecystokinina neurotransmitter, this time secreted in a smaller than necessary amount for the growing thin diet, with lack of that cholecystokinetic food from the diet leading to vesicular hypotonia and thus initiating the vicious circle, therefore, it could be explained the appearance of those cholecystic affections being subsequent to the diet; those diets were statistically proved as risk factors in the appearance of the gallbladder pathology (Morgan, 1991).

Taken into account this risk factor within the growing thin diet as leading to the appearance of the biliary pathology, it was suggested a very balanced food diet where the cholecystokinetic food was not absent. However, not all the persons
bearing a smaller or bigger cotidian stress, or having an unbalanced cholecystokinetic food diet, were to develop a biliary or acute or chronic biliary-pancreatic pathologies. The explanation leads also to the neurotransmission mechanism and we know that each neurotransmitter had its specific cell receptor. Then, as we know that the large number of receptors on the cellular area is part of the program genetically determined [matrix-cell relationship was responsible for keeping that number and their distribution upon the cell area (Gumbiner 1999)].

Cholecystitis or acute cholecystopancreatitis appeared suddenly postoperatory, after laborious surgical interventions could have more intricately explanations: operatory stress determining increased secretion of adrenergic or dopaminergic type neurotransmitters, that would determine an increased contractility into the vesicular wall with the initiation of a vicious circle with vascularization disorders and matrical elements secretion in a short time, edema (as it was mentioned above), resorption disorders, etc.; on the other part, cholecystokinetic food absence given by the prolonged parental feeding, would emphasize vesicular hypotonia reached by means of the vicious circle after a prolonged contraction creating, in turn, another vicious circle continuing to affect both the gallbladder features by resorption disorders and the matrix-cell functionality; it could be also added the experimentally proved fact (Anne Wood 2001; Ligong 2004) that, the prolonged treatment (given to the patients with great surgical interventions) with heparine, heparinases and chlorurs would remove the glycosaminoglycanic chains of the transmembranary proteoglycan ectodomain, by competition: syndecans (intercommunication between them being some of the intracell signal transmitter receptors) thus decreasing the matrixits afferent cells adhesion, therefore, initaiting a balance breaking process of functionality in interdeterminism of the matrix and cells, emphasizing all the complicated enough processes which were initiated by the phenomena briefly mentioned above, phenomena which, extremely simple viewed, belonged to some entire complicated processes starting, developing and keeping up to each other as in a cascade.

Conclusions

1. Extracellular matrix together with the cells afferent to him from the gallbladder wall structure, represents an anatomic and functional unitary whole in the structures determinism from the cholecyst orthology and pathology.

2. Activation or inactivation of the extracellular matrix elements synthesis by endo-and/or exogenous factors leads to structural changes into the cholecyst wall and to the initiation of microanatomic changes inducing vesicular wall disfunction and activating the bile-resorption disfunctionalities and the vesicular wall contractility cascade, thus initiating a vicious circle of appearing, mentaining and/or advancing the pathologic process, leading to favourable conditions for bile nucelation and lithogenity.

3. Extracellular matrix from the gallbladder wall and the cells afferent to the former functions as an unitary whole, microanatomically, the component structures of the vesicular wall (vessels, nerves, cells) are anchored among them by means of the matrical elements surrounding them and determining their function as a whole, influencing the cholecyst functionality.

4. “Neuromuscle units” present into the cholecyst structure could induce morphofunctional disorders into the cholecyst wall during the cotidian and food stress and according to the number of the membranary receptors of the patient (genetically determined) they could induce chronic and/or acute gallbladder pathology.

Acknowledgements: to Professor Gheorghe S. Dragoi, Md., PhD., Member of the Medical Sciences Academy of Romania for helping me in achieving the present research work and to Profesor Dr. Carmen Ardelean from „Victor Babes“ Institute of Bucarest

References


---

**Corresponding Adress:** Lecturer Ileana Dincă, MD, PhD, Department of Human Anatomy, University of Medicine and Pharmacy of Craiova, Department of General Surgery, County Emergency University Hospital of Craiova E-mail: nadia_dinca@yahoo.com