Stress in cholecyst pathology

ILEANA VIORICA DINCĂ

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

ABSTRACT Chronic or acute daily stress, postoperatory 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, spatial 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 methilen to reveal the structural conjunctive; we used Van Gieson staining to reveal collagenic structures and Gömöri 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 mastocyt-macrophage line; epithelial cells from the mucous tunic of the cholecyst was revealed by the monoclonal antibody CD34 made by DACO; 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 spatial orientation: longitudinal, oblique and circular, giving the impression of a plexiform spatial disposition of the three muscle layers, classically described (*figure nr.1B*); they were closely joined by picrofuxynophyle collagen fibres following their trajec. Into the interfascicular spaces, we easily noted the presence of arterioles and perimyocytary meta-arterioles closely following the muscle fascicles trajec (*figure nr.1A,B*); they were also sectioned in different planes.

![Figure 1](image)

**Figure 1 – Alternating spatial orientation of muscle fascicles: circular and oblique. In the interfascicular spaces is easily noted the presence of perimyocytic meta-arterioles and capillaries. The external tunic contains a lax stroma rich in collagen fibers that are colored by picrofuxine, with spiraled course. 1. Crypts; 2.Creases; 3. Oblique and longitudinal muscle fascicles in the muscular tunic; 4.Serosal tunic, Stain: Van Gieson (A; B), eyepiece: 7, obj. 10 (A; B); x70 (A; B). Case number 142 B. G. 70 Y.O.; Chronic lithiasic colecistitis.**

We also observed that the vessels muscle tunic had direct relationships to the perivascular fibroconjunctive tissue and could also noted that the perivascular 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 perivascular matrix, thus making the vessel lumen remain largely opened (*figure nr.3A*).

![Figure 2](image)

**Figure 2: Re-permeated arterial thrombosis in the vesicular wall (A); Old re-permeated venous thromboses (B); Perivascular stroma with intense angiogenesis. 1.Thromosed artery; 2.Repermeated arterial thrombus; 3.Thromosed vein; 4.Repermeated venous thrombus; 5.Perivascular angiogenesis process. Stain: Hematoxilin-Eosin, (A; B); eyepiece: 7, obj. 5(A), obj. 10(B); x35(A); x70(B). Case number 78 C. M., 67 Y.O., Chronic lithiasic colecistitis.**

There existed the possibility that the three layers of smooth muscle fibres classically described (longitudinal, oblique, circular) 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 detrussor) 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 perivascular winding trajec, 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.

Figure 3: The extracellular matrix of the lamina propria has relations with the epithelium of the mucousa, formed by ciliated cylindrical cells that make up the conjunctive spindles of the mucousa; the blood vessels are surrounded by fascicles of collagen fibers that create anchoring arches for the vessels to the muscular tunic. 1. Lamina propria; 2. Muscular tunic; 3. Venule; 4. Arteriole; 5. Obliquely cut nerve; 6. Mucosal epithelium. Stain: Van Gieson; eyepiece: 7; obj. 10(A); obj. 20(B); x70 (A); x140 (B). Case number 20 D.L. 53 Y.O.; Chronic parahydatid colecistitis.

The study of the extracellular matrix elements classified those fine collagen fibres as being collagen IV belonging to the collagen family FACIT (Prokop 1995; Ricard Bloom, 2000) having an anchoring role of the vasculo-nervous structures to the fibrillar collagen of the extracellular matrix (figure nr.4 A,B). It was also noted that fascicles of conjunctive fibres around the nervous fillet 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).

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 nervous fillets intersected in variable planes, thus achieving a plexiform network with rhomboidal meshes crossed by fine meta-arterioles (figure nr.5B).

Figure 4. In the examination with 20x and 40x objectives, numerous isolated collagen fibers(1) or in groups (2) crease and split into compartments the nerve on a transverse section. At the edge of the nerve, the presence of collagen fiber fascicles is observed, that have a sinusoidal course (3) that bide with collagen fibers that partition the nerve (4) and fine collagen fascicles that seem to anchor the nerve (5). Stain: Van Gieson; eyepiece: 7; obj. 20 (A); obj. 40 (B); x140 (A); x280 (B). Case number 20 D.L. 53 Y.O., Chronic parahydatid colecistitis.

Figure 5. The muscular tunic. Transverse cut through nerves (1) and obliquely through a nervous branch (2) and two muscular cell bundles (3). The extracellular matrix is lax and crossed by numerous meta-arterioles (4). In the examination with the 40x objective, myocyte bundles intercept at various angles, creating a plexiform network with rhomboid spaces. Stain: Van Gieson; eyepiece: 7; obj. 10(A), obj. 40(B); x70 (A); x280 (B). Case number 20 D.L. 53 Y.O.; Chronic parahydatid colecistitis.
On a Gömöri silver impregnation we noted that collagen fibres crossing the miocyte fascicles had continuity relationships to the collagen fibres from the extracellular 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).

Figure 6: The muscular tunic of the gallbladder contains neuromuscular units (1). In the structure of the smooth muscle, networks of argirophyllic collagen fibres (2) appear, which make a plexiform network with small spaces, connected to a neurofibrillary network of the cut nerve fibres in several planes: transversal (3) or obliquely (4). Stain: Gömöri; eyepiece: 7; obj. 10; x70. Case number 20 D. L. 53 Y.O.; Chronic parahydatid colicystitis

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).

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 stereodistribution within the nervous fillet. 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 fascicle 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 wall-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 neurofibrills; 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 fibrilar “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 perimiocytary 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-thought it seemed to be secreted by all the neurons (Virgil Dinca, 2005; RaduSuciu, 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, intracell 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 paracellulary increase or decrease. The presence of the argentaffine materials in those intercellular spaces was a proof of the matrical cholinergic type elements also disposed into the intercellular spaces, as a component part of the desmososomal 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; Ischirom 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 affecion 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 postoperatory ones, taking into account the stereodistribution of the microanatomic and matrical 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, matrical elements hyperproduction, resorption disorders at the level of the cholecyst mucosa; stress persistence and intensity didn’t allow recovering by matrical 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 give 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.

Figure 8: Acute cholecystitis. Agiogenesis (1) in the wall of the cholecyst (A); Duplicated epithelial basement membranes; Activation of the precursor cells (2), intense processes of metaplasia and renewal (3) (B). Stain: immunohistochemistry: CD34 monoclonal antibody for endothelial cells (A); for type IV collagen (B). Case number 8, C.C. 20 Y.O.; Chronic lithiasic colecistitis.

Structurally, necrobiosis with acute inflammatory infiltrate zones appeared (mastocytes and macrophages motility and migration were lightened by matrical 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 cytokinin 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 membranae 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 matrical elements but also that the matrix, by feed back methods, could not manage to “command” the cell secretion of matrical elements to decrease and that the metaloproteinases (MMPs) have been activated, more than that, the metaloproteinases 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 matrical 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 cholecystopancreatic 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 cholecystitis (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 and 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, heparinas 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 matrix- its afferent cells adhesion, therefore, initiating 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. Extracell 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 extracell 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 nucleation and lithogenity.

3. Extracell 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 morfounfunctional 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 Professor Dr. Carmen Ardelean from „Victor Babes” Institute of Bucharest.
References

17. Radu Suciu, Mihai Ardelean:Neurobiologia tulburării de panică. Revista Psihiatri nr. 12, III, 2008;

Corresponding Adress: : Lecturer IleanaDincă, 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_dinc@yahoo.com