

HISTOLOGICAL ALTERATIONS OF GALLBLADDER MUCOSA AND SELECTED CLINICAL DATA IN YOUNG PATIENTS WITH SYMPTOMATIC GALLSTONES

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Histological lesions of gallbladder were described mainly in older patients with cholelithiasis (CH). The aim of the study was to analyse morphological alterations in gallbladder mucosa and selected clinical data of young patients with CH. The studies were conducted on 57 patients with CH, subjected to cholecystectomy in the years of 2003-2007. In course of the years, 37 respective young patients (below 25 years of age) were operated. The comparative group included twenty 50-year-old patients with gallstones. The inflammatory activity (grading) was evaluated using a semiquantitative scale on HE-stained gallbladders. In either group, women with chronic cholecystitis and multiple gallstones prevailed. Histological alterations in young patients involved absence of evident epithelial metaplasia traits, low number of foamy cells and prevalence of eosinophils in gallbladder mucosa. Even if a similar grading in gallbladder walls was noted in young and older patients, only in the former ones, a higher grading was detected in patients with an acute clinical course of the gallstone disease. The results point also to a potential role of local accumulation of eosinophils in gallbladder mucosa in pathogenesis of CH in young patients.

Key words: cholelithiasis, young patients, histopathology, clinical data.

Introduction

Cholelithiasis (CH) belongs to civilization diseases and ten to fifteen per cent of white adults in developed countries harbour gallstones [1]. The factors associated with a high risk of CH continue to include female gender, obesity, insulin resistance, genetic conditioning and age [2-4]. Cholelithiasis is manifested more frequently in older individuals, although in recent years an increase has been observed in incidence of a symptomatic CH in children and young adults [5, 6]. In children and youth, intra- and post-surgery complications of CH were noted more frequently and were associated with accompanying diseases, such as inborn defects of the heart, haemolytic anaemias, hereditary spherocytosis, sickle cell anaemia, mucoviscidosis, long-term parenteral alimentation and other pathologies [7, 8]. Studies on

the youth demonstrated that the most frequent factors of CH risk included female gender, alimentary habits, obesity and disturbances in the liver function [6]. Descriptions of histological lesions in CH are related mostly to adult patients. As a rule, CH is accompanied by acute or chronic cholecystitis in its multiple variants [9]. Chronic cholecystitis is associated with thickening of the gallbladder wall, hyperplasia of muscularis layer, incrustation with gallstones, less frequently with a diffuse calcification of the gallbladder wall ("porcelain gallbladder") [10, 11]. Varieties of chronic cholecystitis include the so-called eosinophilic cholecystitis (EC) [12, 13]. Microscopic lesions of the mucosa include alterations of gallbladder epithelium, its *lamina propria*, with an infiltrate of mononuclear cells of variable intensity and traits of fibrosis. Either normal epithelium or its atrophy, hyperplasia or dysplastic lesions were

reported. Presence of goblet cells was noted (intestinal type of dysplasia) or the epithelium resembled that in the pyloric portion of the stomach (antral type of dysplasia) [14]. The epithelium might include Paneth cells or endocrine cells [15, 16]. Development of metaplastic lesions correlated with age of the patients [17]. Interestingly, the histopathological lesions in gallbladder mucosa (mainly chronic inflammation) were described also in a high number (>30%) of "control" gallbladders and in 100% of gallbladders containing asymptomatic gallstones in their lumen [18]. In another report, no significant morphological lesions were detected in gallbladders sampled on autopsy [19]. Review of the literature indicated that clinical diagnosis of chronic cholecystitis cannot be identified with the respective morphological diagnosis [10]. Moreover, according to many authors, morphological diagnoses such as minimal, mild chronic cholecystitis and normal gallbladder are inaccurate. With the aim of making a more objective assessment of histological alterations in cases of chronic cholecystitis, their investigators attempted to employ semiquantitative scales of inflammatory activity (grading) and of advancement in fibrosis (staging) [19, 20]. Manifestation of CH in young patients understandably induces interest since causes of CH most frequently remain unknown and the problem is increasingly urgent. Better techniques are searched for earlier diagnosis, therapy and, first of all, prevention of CH in this age group [5]. No detailed descriptions are available of histopathological lesions in gallbladders of young patients with symptomatic CH.

In this study, we decided to perform an analysis of gallbladder morphological lesions, as correlated with selected clinical data in young patients (up to 25 years of age) with symptomatic CH. For comparison, patients most frequently subjected to cholecystectomy in the same period of time were studied, i.e. patients of approximately 50 years of age with the same diagnosis.

Material and methods

Patients

All the patients were subjected to cholecystectomy in the T. Chałubiński Municipal Hospital in Ostrów Wielkopolski, Poland. Group A (n = 37; young patients) included all patients up to 25 years of age (16 to 25 years of age, 31 women and 6 men) who were diagnosed and subjected to surgery in years 2003-2007. For the comparative group (group B), the age criterion was accepted, amounting to approximately 50 years as this was the most frequent age of cholelithiasis patients subjected to surgery at the Surgical Ward of the Municipal Hospital. Twenty

patients were selected to the group (48 to 50 years of age, 15 women and 5 men). The duration of CH symptoms in the analysed groups of patients most frequently ranged between 6 months and one year.

In group A, the histopathological diagnoses in hospital records were as follows: 35/37 (94.6%) patients exhibited traits of chronic cholecystitis, in two patients (5.4%) acute cholecystitis was diagnosed, including one case with partially purulent and one case with gangrenous acute cholecystitis. In group B, all the patients demonstrated histopathological diagnosis of chronic cholecystitis. The available epidemiological data included: age, gender, in women: number of pregnancies/deliveries, results of laboratory tests, data related to anamnesis [symptoms of the disease directly before surgery (acute or chronic pain) and duration of symptoms, coexisting chronic diseases (arterial hypertension, diabetes mellitus)], body temperature (BT) and body mass index (BMI) upon admission to the hospital. The number of gallstones revealed in cholecystectomy specimens was estimated using a semiquantitative scale: 1 – a single gallstone of any size; 2 – 2 to 20 gallstones; 3 – 21 to 100 gallstones; 4 – > 100 gallstones. Written informed consent was obtained from each patient before operation, and approval for the study was granted by the institution's Ethical Committee (No. 281/08).

Tissue preparation and microscopy image analysis

The tissue material included gallbladders and for light microscopy, tissue specimens were fixed in buffered 10% formalin and embedded in paraffin using the routine procedure. The studies were conducted on serial, 5 μ m paraffin sections, placed on the microscopical slides. Histopathological lesions were evaluated following the classical hematoxylin and eosin (HE) staining. Patterns of HE-stained histological preparations were examined using Olympus B2 light microscope coupled to a digital camera by two histopathologists (WB, AK). The analysis included detailed description of histopathological alterations related to simple columnar epithelium and *lamina propria* of the mucosa. Each tissue specimen was also evaluated based on a simple numerical scoring system for the grade of *lamina propria* inflammation (G1) (0-3), the grade of muscularis externa/adventitia inflammation (G2) (0-3) and the final grading (G1 + G2), in which 3 points denoted intense and most frequently diffuse inflammatory infiltrate, 2 points referred to moderately intense but also diffuse inflammatory infiltrate, 1 point indicated individual, dispersed cells or focally arranged cells of inflammatory infiltrate, 0 points indicated tissue sections in which no inflammatory cells could be detected. Thickness (width) of the total wall of gallbladder was measured in mm.

Statistical methods

The parameters of descriptive statistics (arithmetic mean, standard deviation, median value, minimum and maximum value) were calculated. Results of studies were compared between groups A and B (unlinked samples) using the test of Mann-Whitney. Correlations between data rows were determined employing Spearman's rank correlation index. The test for two structural indices was used to evaluate differences between fractional detectability of selected traits between the groups. Statistically significant relationships or differences were diagnosed at the significance level of $p \leq 0.05$. The statistical analysis was conducted using the Statistica PL v.7.1 software (Statsoft, Inc.).

Results

Selected clinical data

Mean age of the young patients was 21.4 ± 2.5 years and it was significantly different from the patients' age in group B (49.1 ± 1.2 years) ($p = 0.001$). In either group, women prevailed. The older age group included a significantly higher number of obese patients ($BMI \geq 30$) than in group A (Table I and II).

The older patients more frequently reported arterial hypertension as a coexisting disease. Only 3 patients of group B suffered from diabetes mellitus. Numbers of deliveries did not differ significantly between women in the two groups. At the time of admission to the hospital, significantly more young patients reported chronic symptoms and only 12 of them (32%) demonstrated acute clinical course at admission. In older patients, no significant differences were disclosed in the number of patients with acute vs. chronic course of the disease (Table I). In either group, a significantly higher number of multiple gallstones were observed in comparison with solitary ones (Table I). A significantly higher WBC count was noted in group B patients vs. group A patients. No differences between the groups were observed in the number of blood platelets (PLT), haemoglobin content or in the level of total bilirubin. On admission, mean BT remained within normal limit in both groups (Table II).

Histopathological findings

Young patients (group A)

Histopathological diagnoses set upon routine hospital diagnosis were confirmed: 35/37 (94.6%) patients exhibited traits of chronic cholecystitis. In two

Table I. Epidemiological and clinical data of young (A) and older patients (B) with CH

PARAMETER	GROUP A	GROUP B	P
Women	31 (84%)	15 (75%)	0.413
Men	6 (16%)	5 (25%)	0.414
Obesity	5 (14%)	11 (55%)	0.002
Pregnancy/deliveries	26% of women	33% of women	0.624
Arterial hypertension	5 (14%)	12 (63%)	0.001
Acute course of the disease	12 (32%)	10 (50%)	0.188
Chronic course of the disease	25 (68%)	10 (50%)	0.188
Solitary gallstones	9 (24%)	6 (30%)	0.624
Multiple gallstones	28 (76%)*	14 (70%)**	0.624

The table provides parameters expressed as a number of cases with % in brackets; p – level of significance; *p = 0.001 between solitary and multiple gallstones in group A; **p = 0.016 between solitary and multiple gallstones in group B

Table II. Selected clinical data, results of laboratory tests in young (A) and older (B) patients with cholelithiasis (mean \pm SD)

PARAMETER	GROUP A	GROUP B	P
BT at admission ($^{\circ}$ C)	36.69 ± 0.35	36.62 ± 0.22	0.215
BMI (kg/m^2)	25.38 ± 4.03	29.16 ± 4.01	0.001
Haemoglobin (g/dl)	13.63 ± 1.31	13.97 ± 1.87	0.140
White blood cells (WBC; $\times 10^9/\text{l}$)	8.20 ± 3.43	11.10 ± 4.55	0.016
Platelets (PLT; $\times 10^9/\text{l}$)	229.63 ± 46.99	238.60 ± 65.24	0.265
Total bilirubin (mg/dl)	1.73 ± 1.10	0.77 ± 0.31	0.138

BT – body temperature; BMI – body mass index; SD – standard deviation; p – level of significance

patients (5.4%), acute cholecystitis was diagnosed. In most patients of the group, epithelium of gallbladder was normal, it covered *lamina propria* and protruded with it in the form of villus-like folds to the lumen of gallbladder. Rokitansky-Aschoff sinuses with deep penetration of muscularis layer and moderate hypertrophy of muscularis externa were observed in 10/37 (27%) patients. The epithelium represented a typical simple columnar epithelium with lucid cytoplasm and normal cell nucleus in the basal parts of the cells, on the surface covered with mucus and occasionally containing biliary deposits in the lumen (Fig. 1A). Presence of intraepithelial lymphocytes, neutrophils and/or mast cells was observed (Fig. 1B). Foci of epithelial regeneration were seen, with cuboidal or flat cells and a typical structure of cell nuclei. Few goblet cells were present in a single fragment of the epithelium in one patient. In the entire tissue material, the typical connective tissue of *lamina propria* with a well-developed network of blood vessels was characteristic. Single high endothelial postcapillary venules were observed in 9/37 (24%) patients. Among inflammatory infiltrate cells, lymphocytes, eosinophils, mast cells, monocytes/macrophages and neutrophils were noted (Fig. 1B). In 7/37 (19%) patients, solitary lymph

nodules were present. In 10/37 patients (27%), eosinophils prevailed among cells of inflammatory infiltrate ($\geq 80\%$) and were very numerous (Fig. 1C). The cells were interspersed with few lymphocytes, macrophages/monocytes and proliferating fibrocytes. Inflammatory infiltrates of a similar cell content (including eosinophils) were present also in the remaining layers of the gallbladder wall. In 2/37 (5%) patients, hyperplastic tubuloacinar mucous glands with typical traits of hyperplasia adenomyomatosa in *lamina propria* were observed (Table III).

Older patients (group B)

As compared to group A, more frequently the epithelium manifested fragments with intense damage and regenerative reaction with cuboidal and/or flat cells but with normally stained cell nuclei (Fig. 2A). In 4/20 patients, goblet cells in the epithelium (in one patient very numerous) were found (Fig. 2B) (Table III). In one woman, more extensive lesions in epithelium were noted (abnormal and irregular regeneration of the epithelium, with polymorphism and polychromasia of cell nuclei, numerous fields of neutrophils) with rich subepithelial neutrophilic infiltrate. Numerous Rokitansky-Aschoff sinuses

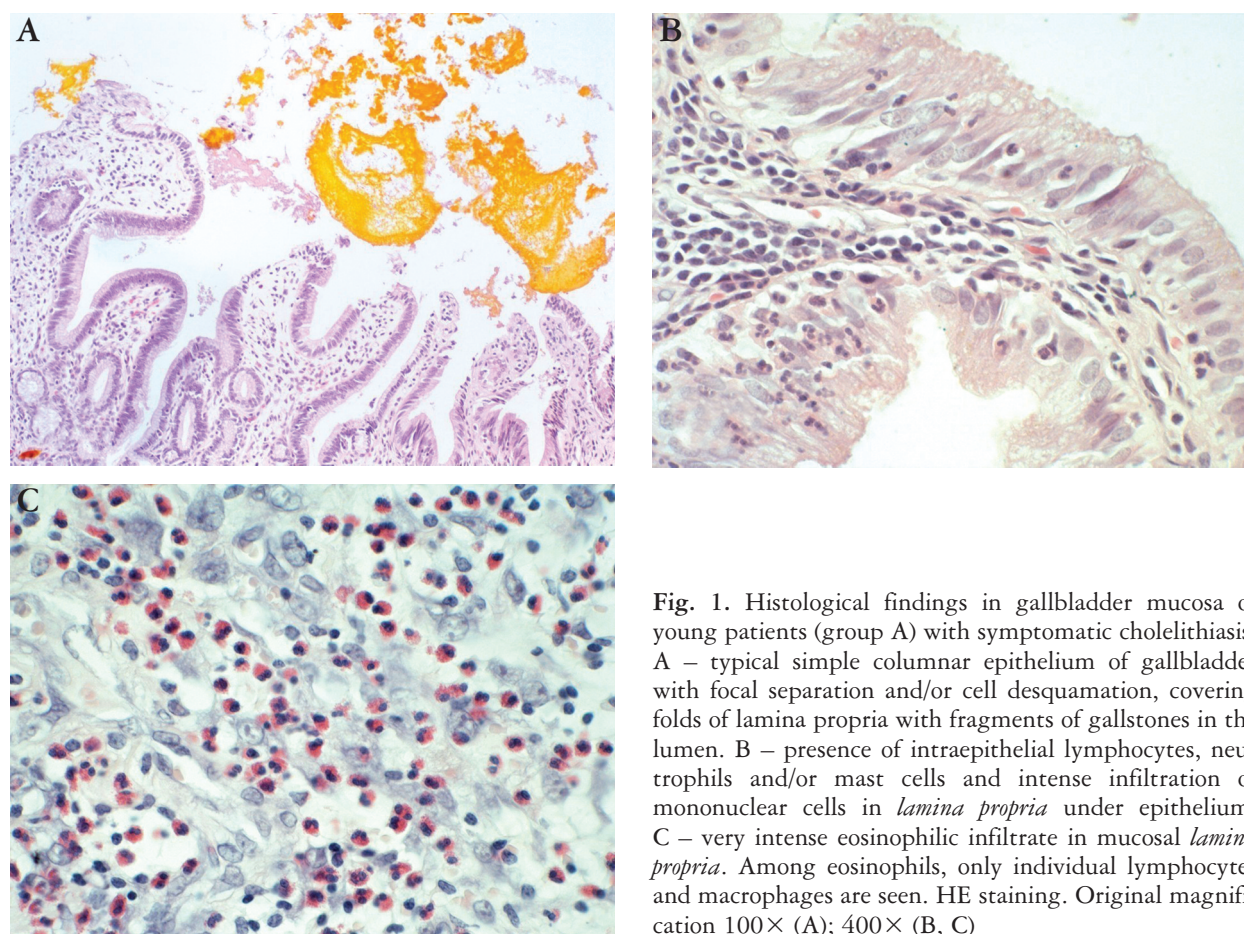


Fig. 1. Histological findings in gallbladder mucosa of young patients (group A) with symptomatic cholelithiasis. A – typical simple columnar epithelium of gallbladder with focal separation and/or cell desquamation, covering folds of lamina propria with fragments of gallstones in the lumen. B – presence of intraepithelial lymphocytes, neutrophils and/or mast cells and intense infiltration of mononuclear cells in *lamina propria* under epithelium. C – very intense eosinophilic infiltrate in mucosal *lamina propria*. Among eosinophils, only individual lymphocytes and macrophages are seen. HE staining. Original magnification 100 \times (A); 400 \times (B, C)

Table III. Frequency of manifestation for repeatedly noted histopathological lesions in patients with CH

HISTOLOGICAL FINDINGS IN GALLBLADDER MUCOSA	GROUP A	GROUP B	P
intense epithelial regeneration	7 (19%)	7 (35%)	0.186
goblet cells in the epithelium	1 (3%)	4 (20%)	0.036
vast infiltrates of foamy cells (cholesterolosis)	2 (5%)	6 (30%)	0.012
intense infiltrates of eosinophils*	10 (27%)	1 (5%)	0.049
hyperplastic tubuloacinar glands	2 (5%)**	5 (25%***)	0.027
lymph nodules in <i>lamina propria</i>	7 (19%)	8 (40%)	0.092
high endothelial venules (HEV)	9 (24%)	4 (20%)	0.731

The table provides parameters expressed as a number of cases and % in brackets;

* $\geq 80\%$ of cells in the inflammatory infiltrate; ** both with adenomatous hyperplasia; *** with adenomatous hyperplasia in 2 of them; p – level of significance

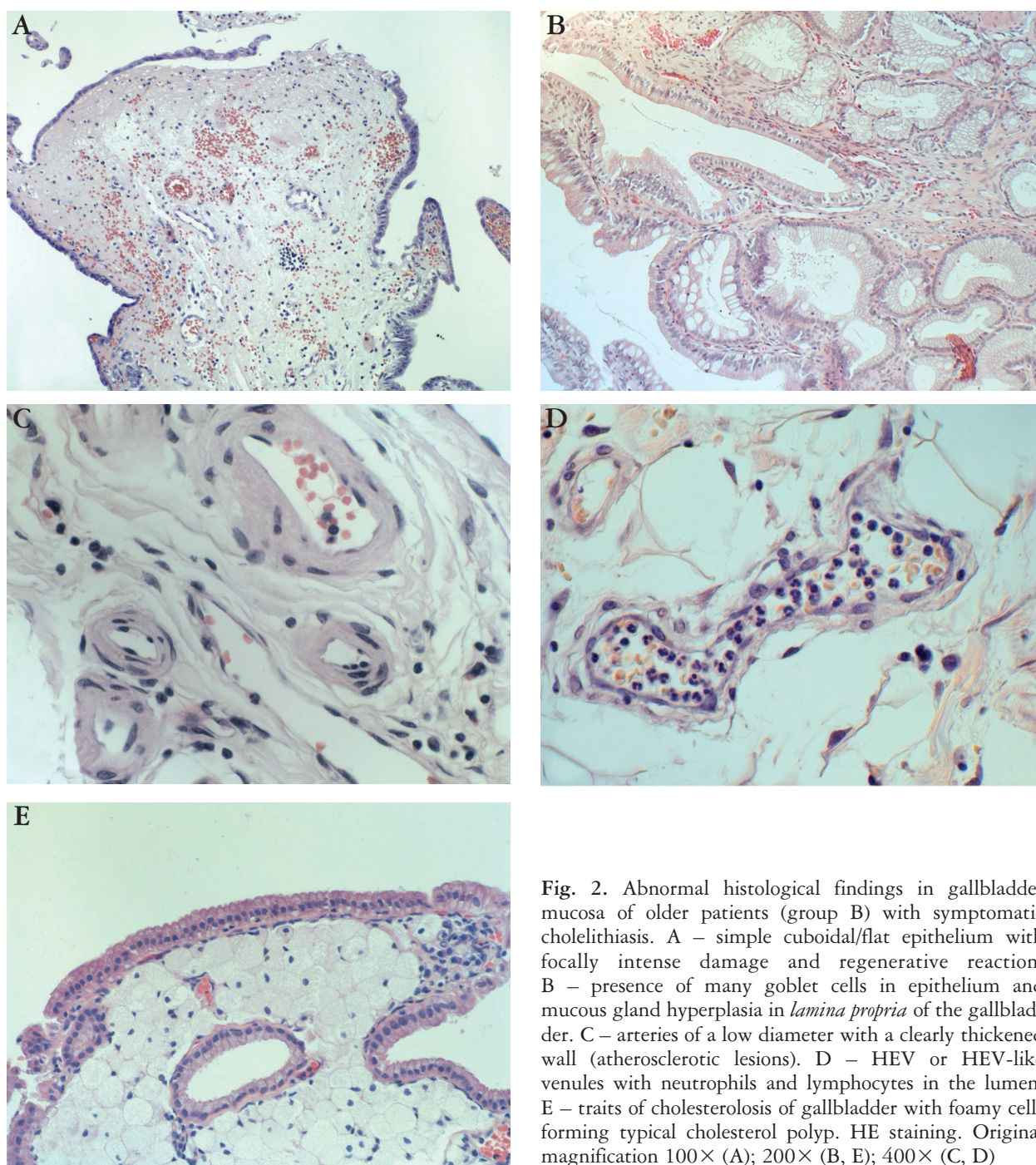


Fig. 2. Abnormal histological findings in gallbladder mucosa of older patients (group B) with symptomatic cholelithiasis. A – simple cuboidal/flat epithelium with focally intense damage and regenerative reaction. B – presence of many goblet cells in epithelium and mucous gland hyperplasia in *lamina propria* of the gallbladder. C – arteries of a low diameter with a clearly thickened wall (atherosclerotic lesions). D – HEV or HEV-like venules with neutrophils and lymphocytes in the lumen. E – traits of cholesterolosis of gallbladder with foamy cells forming typical cholesterol polyp. HE staining. Original magnification 100 \times (A); 200 \times (B, E); 400 \times (C, D)

were noted in all except for two patients (90%). Connective tissue of *lamina propria* was very well supplied with blood vessels, frequently manifested a significant fibroplasia and/or oedema. Blood vessels of a low diameter with atherosclerotic lesions were repeatedly encountered and in most patients of the group (Fig. 2C). The HEVs were noted in 4/20 patients and, thus, in a similar proportion of patients to that in the younger group (Fig. 2D). Gallbladder cholesterosis with foamy cells was detected in 6/20 patients, more frequently than in group A (Fig. 2E). Inflammatory cells (macrophages/monocytes, lymphocytes and mast cells) were frequently present directly below epithelium. Except for a single patient only, individual eosinophils were present in the inflammatory infiltrates. In 5/20 patients hyperplastic mucous tubuloacinar glands with traits of adenomatous hyperplasia were found (Table III).

Correlations between inflammatory activity (grading) and selected clinical data

Young patients (group A)

Neither mean grading in *lamina propria* (G1), nor grading in deeper layers of the gallbladder wall (G2) or the total grading (G1 + G2) significantly differ from these parameters in group B (Table IV). However, in this group of patients, higher G2 was detected as compared to G1 (Table IV). Significantly higher grading (G2 and G1 + G2) was noted in young patients with acute clinical symptoms in comparison

with the patients with chronic complaints before surgery (Table V). No significant correlations could be detected between grading (G1, G2, G1 + G2) and the number of gallstones ($r = 0.131$; $r = -0.208$; $r = -0.102$, respectively; $p > 0.05$). Also gallbladder grading did not correlate with WBC number in the group ($r = 0.034$; $r = 0.237$; $r = 0.180$, respectively; $p > 0.05$). No significant differences in the number of gallstones could be detected between the younger and the older patients (Table IV). In the entire group, the number of gallstones manifested a negative correlation with BMI ($r = -0.329$; $p < 0.05$). The mean width of the gallbladder wall was 5.62 ± 2.01 mm and it did not differ from that in group B (Table IV). No significant correlations could be documented between width of the gallbladder wall on one side and patient's age, BMI, number of gallstones and the final grading (G1 + G2) on the other ($r = 0.014$; $r = 0.011$; $r = -0.323$; $r = 0.243$, respectively; $p > 0.05$). Also, no differences in the thickness of the gallbladder wall could be related to clinical symptoms.

Older patients (group B)

Similarly to young patients, more intense inflammatory lesions were detected in deeper layers of the gallbladder wall (G2) as compared to grading in *lamina propria* (G1) (Table IV). G1 manifested a direct relationship with G2 and with the final grading (G1 + G2) (Spearman's correlation index $r = 0.529$; $r = 0.896$; respectively; $p < 0.05$), also

Table IV. Comparative analysis of grading, number of gallstones and width of the gallbladder wall in young and older patients with cholelithiasis (mean \pm SD)

PARAMETER	GROUP A	GROUP B	P
Grading 1 (G1)	1.45 \pm 0.64	1.55 \pm 0.75	0.672
Grading 2 (G2)	1.86 \pm 0.71*	2.00 \pm 0.64**	0.512
Final grading (G1 + G2)	3.32 \pm 1.08	3.55 \pm 1.23	0.567
Number of gallstones	2.37 \pm 1.11	1.95 \pm 0.82	0.241
Width of the gallbladder wall (mm)	5.62 \pm 2.01	5.51 \pm 2.01	0.810

SD – standard deviation; p – level of significance; *p = 0.019 between G1 and G2 in group A; **p = 0.022 between G1 and G2 in group B

Table V. Analysis of grading in patients with CH as related to acute/chronic symptoms of disease at admission to the hospital (mean \pm SD)

PARAMETER	CLINICAL COURSE	GROUP A	GROUP B	P
Grading 1 (G1)	acute	1.58 \pm 0.66	1.80 \pm 0.78	0.582
	chronic	1.40 \pm 0.64	1.30 \pm 0.67	0.816
Grading 2 (G2)	acute	2.33 \pm 0.65*	2.20 \pm 0.78	0.771
	chronic	1.64 \pm 0.63	1.80 \pm 0.42	0.439
Final grading (G1 + G2)	acute	3.91 \pm 1.16**	4.00 \pm 1.49	0.923
	chronic	3.04 \pm 0.96	3.10 \pm 0.74	0.734

SD – standard deviation; p – level of significance; *p = 0.010 between G2; **p = 0.035 between G1 + G2 in patients with acute and chronic symptoms

G2 showed positive correlation with the final grading (G1 + G2) ($r = 0.830$; $p < 0.05$). No significant differences could be documented in grading (G1, G2, G1 + G2) and acute/chronic course of disease (Table V). Similarly to group A, in the older patients, multiple gallstones were encountered with a significantly higher frequency (Table I). No correlations could be documented between grading (G1, G2, G1 + G2) and the number of gallstones ($r = 0.145$; $r = -0.097$; $r = 0.088$, respectively, $p > 0.05$) and WBC number ($r = 0.190$; $r = 0.192$; $r = 0.145$; respectively; $p > 0.05$) (Table II). In group B, the number of gallstones showed no correlation with BMI ($r = 0.230$; $p > 0.05$). No correlations could be detected between width of the gallbladder wall on one hand and BMI, number of gallstones and final grading ($r = 0.262$; $r = -0.217$; $r = 0.032$, respectively; $p > 0.05$).

Discussion

Among young patients subjected to cholecystectomy in years 2003-2007 analyzed in this study, 37 patients were aged 16-25 years. The group was dominated by women. Obesity was documented in 14% of patients (5/37). The results are consistent with those of other investigators, who linked CH with female sex, but obesity did not seem to pose a significant risk factor for CH in the group of patients [6, 21]. In both groups the majority of patients had multiple gallstones and the number showed a negative correlation with BMI only in young patients. The two groups of patients did not significantly differ from each other in the number of experienced deliveries. Comparison of the number of gallstones in both groups of patients has shown no significant differences. Our study generally confirms histological diagnosis established within the routine diagnosis. In addition, in two cases, cholesterosis of gallbladder was detected. In the group of young patients, routine staining did not disclose typical traits of metaplasia in gallbladder epithelium while in the older patients metaplastic lesions (presence of goblet cells in the epithelium) was detected in 20% of patients. Eosinophils as a dominating type of cells in the inflammatory infiltrate were noted in a great number of young patients (27% of the cases), as compared to a single patient in the comparative group. According to other authors, adult patients with CH demonstrated more pronounced inflammatory lesions as compared to cholecystitis in younger patients without gallstones [22]. Microscopic lesions of mucosa most frequently included various grading and various stage of parietal fibrosis (staging). More advanced fibrosis of the organ and a higher number of inflammatory cells were demonstrated in men as compared to women [23]. Another study demon-

strated that also the "control" gallbladders and asymptomatic cases of CH are associated with morphological lesions, first of all in the mucosa. The histological lesions in the gallbladder were suggested to be linked to advanced age and they were present mainly in female patients [18]. Baig *et al.* detected "pure" chronic cholecystitis in only 50% of the patients with CH [9]. Presence of goblet cells, enterochromaffin cells or antral-type (pseudopyloric) glands in gallbladder epithelium in cases of CH were thought to represent intestinal or gastric metaplasia, and to represent premalignant lesions in the organ [15, 16, 24]. In our study, only in the group of older patients, a single patient has demonstrated evident pathological lesions of the epithelium. This confirms the suggestion that incidence of meta- and dysplastic lesions increases with age and is higher among females [17]. Nevertheless, some reports demonstrated no correlations between the extent of metaplasia on one hand and patient's age, duration of symptoms of the disease or its severity on the other [25]. In the younger group, the prevalence of eosinophils in inflammatory infiltrates has drawn our particular attention. The references indicate that the so-called eosinophilic cholecystitis (EC) is usually manifested in cholecystitis without gallstones and involves presence of $\geq 90\%$ eosinophils in inflammatory infiltrates in gallbladder walls [12, 13]. Nevertheless, clinical cases of idiopathic chronic EC were described also in patients with CH [26]. Some authors regard EC to be extremely rare [27], others indicate the growing number of detected cases of chronic cholecystitis with infiltrates consisting of eosinophils [12]. The quoted papers have not pertained to young patients. Relatively recently EC has been described in a 29-year-old patient with no peripheral eosinophilia [13]. Studies from the 1980s related to eosinophil inflammatory reaction in isolated organs of adult patients have shown that local infiltrates of the cells are encountered more frequently in organs where inflammation is usual and many cases have no allergic history [28]. As compared to other diseases of the alimentary tract, in cholecystitis the role of eosinophils is less known. In EC it is suggested that the disease may be more frequent than earlier thought and that it may affect individuals with a unique or hypersensitivity type of inflammatory response to an altered bile [29]. Examining exclusively the patients with gallstones we are unable to confirm the observation that EC is manifested more frequently in stone-free cholecystitis. Inflammatory infiltrates in gallbladders of our young patients consisted also of lymphocytes, mast cells and, less numerous than in older patients, macrophages. The result is consistent with observations conducted mainly on adult patients [30]. Mast cells containing numerous chemotactic factors may

be responsible for accumulation of also numerous eosinophils in inflammatory infiltrates observed in chronic cholecystitis [30].

The use of a semiquantitative scale permitted to evaluate average intensity of gallbladder inflammation (G1, G2, G1 + G2), the parameters which in young patients manifested low values and, surprisingly, did not significantly differ from analogous grading in 50-year-old patients. Among young patients no direct relationship could have been established between grading in *lamina propria* (G1) and that in deeper layers of the gallbladder wall (G2), the relationship which has been noted in older patients. No significant correlations could have been detected between grading in the gallbladder wall on one hand and WBC number in peripheral blood and the number of removed gallstones. Another study documented a positive relationship between inflammation and the diameter of the largest gallstone and a negative relationship between inflammation on one hand and the number of gallstones and patient's age on the other [31]. In our study, width of the entire gallbladder wall in both groups of patients has shown no correlation with the patient's age, BMI or with inflammatory activity (grading) in the wall of the gallbladder. No differences in thickness of the gallbladder wall could have been linked to acute or less intense clinical course of the disease. We have been able to confirm observations of other authors who demonstrated thickness of the gallbladder wall greater than 3-6 mm in 71% of patients with chronic cholecystitis [32]. The semiquantitative scales for evaluation of inflammation activity in the gallbladder wall used by other authors were most frequently based on 3 or 5 point systems [19, 20]. Independently of the applied semiquantitative method, descriptive methods of grading and/or staging evaluation are used [10, 19, 20, 30, 31]. In 100 adult cholecystectomized patients mild (28%) or moderate (62%) cholecystitis was recognized. In most of the patients with chronic cholecystitis the authors noted traits of epithelial metaplasia and regeneration [19]. In our young and older patients, the grading has resembled the quoted reports but we have not detected such a high proportion of metaplasia as that given by the author (75%), even in the group of older age [19]. This might have reflected the more age-uniform group of our older patients and much lower number of cases than that studied by the authors (20 vs. 100).

Conclusions:

- As a rule, in young patients with cholelithiasis morphological lesions in mucosa did not involve gallbladder epithelium but qualitative and quantitative differences of inflammatory infiltrate in *lamina propria*.
- Even if a similar grading in gallbladder walls was noted in young and older patients, only in the former, a higher grading was detected in patients with acute clinical course of the gallstone disease.
- The decisive prevalence of eosinophils in inflammatory infiltrates in almost 30% of young patients suggests involvement of the cells in pathogenesis in symptomatic cholelithiasis in young patients.

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