

INFLUENCE OF GENDER ON THE INTENSITY OF INFLAMMATIVE RESPONSE IN INFLAMMATORY GALLBLADDER DISEASES

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Abstract

Cholecystitis as an inflammatory reaction is recognized in symptomatic and asymptomatic form with or without complications. The aim is comparison of cholecystitis as an inflammatory process between the male and female population and proving a more intense inflammatory response in the male population.

The study was conducted in the CGH "8th September" in the period 2019-2020. The collected data were analyzed with the statistical program SPSS Version 17.0.

The research included and analyzed a total of 36 (n = 36) respondents who were divided into two groups. The first group of 17 (47.2%) patients are male and the second group of 19 (52.8%) patients are female.

Laboratory tests showed a significant difference between the two tested groups in the measured values of progesterone with a significance of $p = 0.027$ in favor of the female population, and for testosterone and IgA in favor of the male population ($p = 0.001$, $p = 0.036$, respectively), while in other markers examined no significant difference between the sexes was found.

The influence of the hormonal factor on the intensity and character of the inflammatory changes is also shown by the values obtained from the laboratory tests in the study which showed a significant difference between the two groups in the measured values of progesterone with significance in favour of the female population, and for testosterone and IgA in favor of the male population, which proved the influence of the hormonal factor on the intensity and character of inflammatory changes.

Keywords: cholecystitis, cytokines, estrogen, inflammation testosterone.

Introduction

Inflammation of the gallbladder (cholecystitis) is a disease whose surgical treatment occupies the largest percentage in abdominal surgery.

The most common cause of this disease is considered to be the presence of stones (calculosis) in the gallbladder (cholecystolithiasis) but there is also cholecystitis without cholecystolithiasis and cholecystolithiasis without cholecystitis [1].

Inflammation as a reactive process of complex biological response is an immune defense reaction of the organism characterized by a multitude of cascading mutually supportive processes that through the creation and release of a number of mediators such as cytokines (tumor necrotizing factor, interleukin 1,2,6,8), protein structures (CRP, fibrinogen) and in coordination with the participation of hormonal factors, including sex hormones (estrogen and testosterone), is a defense reaction of the organism that in acute or chronic form generically activates the innate or acquired immune system [13, 15-17, 21,22].

Acute inflammation is the initial response of the organism that begins with activation or movement of leukocytes, and above all the granulocyte lineage of the blood, targeting the "attacked" tissue which through a series of biochemical processes activates local (local vascular and tissue changes) and systemic immune responses [14]. Acute inflammatory response as a short-term coordinated systemic and local mobilization response to immune, endocrine, and neurological mediators may develop in a variety of characteristic ways such as serous, granulomatous, fibrin, purulent, and ulcerative inflammation.

The inflammatory process in the form of a continuous recurrent process can take a chronic course marked by varying intensity of fibrous changes.

Cholecystitis as an inflammatory reaction is recognized in symptomatic and asymptomatic form with or without complications.

The consequences of cholecystitis can range from a mild form of inflammatory process (acute and chronic) to serious changes such as acute progressive cholecystitis (hydrops, empyema, gangrene, perforation), choledocholithiasis with or without cholangitis, jaundice, pancreatitis, a fistula between the gallbladder with a biliary canal or organ (ductus choledochus, duodenum, small intestine and stomach), ileus, cancer, and lethal outcome [2,3].

Numerous scientific studies and analyzes have irrefutable evidence that inflammatory gallbladder disease in men is less common compared with the female population, but the severity of the inflammatory process, the complications of the inflammatory process itself, the higher incidence rate of conversion from laparoscopic to open method of cholecystectomy are significantly larger in the male population than in the female population [4-7, 18].

The hormonal factor in many other diseases as well as in gallbladder inflammation has an impact on the severity of inflammatory changes.

The influence of estrogen and testosterone in reducing or increasing the severity of inflammation is an important factor in the evolution of the inflammatory process and its complications [8,9].

In terms of proinflammatory cytokines at the tissue level, there is a difference between men and women with cholecystitis [10-12,17].

Materials and methods

The study was conducted in the CGH "8th September" in the period 2019-2020, in the departments of abdominal, general and emergency surgery, department of pathology, department of laboratory and biochemical analysis and department of gastroenterohepatology.

Laboratory analyses were made on postoperatively taken blood samples, and the histopathological analysis on a tissue sample of a surgically removed gallbladder. In terms of design, the research is a retrospective intervention study.

The contingent of respondents in the study consisted of 36 patients who were divided into two groups. The first group of 17 respondents were patients operated on from the male population, and the second group of 19 patients operated on from the female population.

Inclusive criteria: Patients treated as emergencies where acute calculous and acalculous cholecystitis has been diagnosed, patients treated as elective cases diagnosed with chronic calculous and acalculous cholecystitis, patients aged 16 to 80 years.

Exclusive criteria: patients with other gallbladder pathology (malignancies, congenital biliary tract abnormalities), patients with comorbidities that are a contraindication to surgical treatment.

Research data were provided from disease histories, operative protocols, results obtained from histopathological analyzes, and the HIS hospital information system.

The collected data were analyzed with the statistical program SPSS Version 17.0. The numerical signs were presented with an arithmetic mean with standard deviation, the attributive ones with frequency distributions.

Fisher exact test, Student's t test for independent samples, Mann-Whitney test were used to compare male and female patients in relation to the analyzed variables. Values of $p < 0.05$ were taken as statistically significant.

Histopathological analysis was performed on the principle of delivered operative material from cholecystectomy previously fixed for at least 24 hours in 10% formaldehyde solution. From the material were taken 1-3 samples in length of 1-2 cm from previously macroscopically assessed zones with pathological changes.

The samples were processed by automation process in a Thermo Fisher Scientific Citadel 2000 tissue processor, and then molded into paraffin molds.

From the material in the paraffin molds, tissue sections with a thickness of 4 microns were made, which after the process of deparaffinization were stained with H&E in an automated stainer Thermo Fisher Scientific Gemini AS. The sections were analyzed on a Nikon Eclipse E600 light microscope.

Results

The research included and analyzed a total of 36 ($n = 36$) respondents who were divided into two groups. The first group of 17 (47.2%) patients are male and the second group of 19 (52.8%) patients are female. Male and female patients did not differ in age ($p = 0.6$). Male patients were insignificantly older than female patients (57.76 ± 14.4 vs. 55.63 ± 15.4).

As a surgical technique in a total of 36 patients, in 29 (80.5%), the method of laparoscopic cholecystectomy approach was used, in 5 cases the open (14%) method, while the conversion from laparoscopic to open method was used in 2 (5.5%), i.e. the percentage prevalence in the male group of patients treated with laparoscopic method was 14 (82.3%), open method in 2 (11.8%) and conversion from laparoscopic to open method was applied in 1 (5.9%) patient.

In the female group, laparoscopic approach was applied in 15 (78.9%), open method in 3 (15.8%) and conversion from laparoscopic to open method was applied in 1 (5.3%).

The results in table 1 showed that there was no significant difference between the sexes in terms of the application of the surgical technique as well as the conversion from laparoscopic to open method of cholecystectomy.

Table 1. Type of surgical technique in relation to gender.

Type of surgical technique	Gender of respondents		p value
	Male	Female	
	n (%)	n (%)	
Laparoscopic approach	14 (82.3)	15 (78.9)	p=1.0 ns
Open approach	2 (11.8)	3 (15.8)	
Laparoscopic to open conversion	1 (5.9)	1 (5.3)	

p (Fisher exact)

The results obtained from the conclusion on the nature of the inflammatory process defined in the operative protocol and the list of histopathological analysis showed that out of a total of 36 ($n = 36$) patients, in 8.3% ($n = 3$) an acute inflamed gallbladder was verified, and in 91.7 % ($n = 33$) chronically inflamed gallbladder.

Regarding the gender, it was shown that in the male group of respondents 15 (88.2%) were chronic, and 2 (11.8%) with acutely inflamed gallbladder, while in the female group chronically inflamed gallbladder was found in 18 (94.7%) patients, and acute in 1 (5.3%), and without statistical significance (Fisher exact, $p = 0.46$). Figure

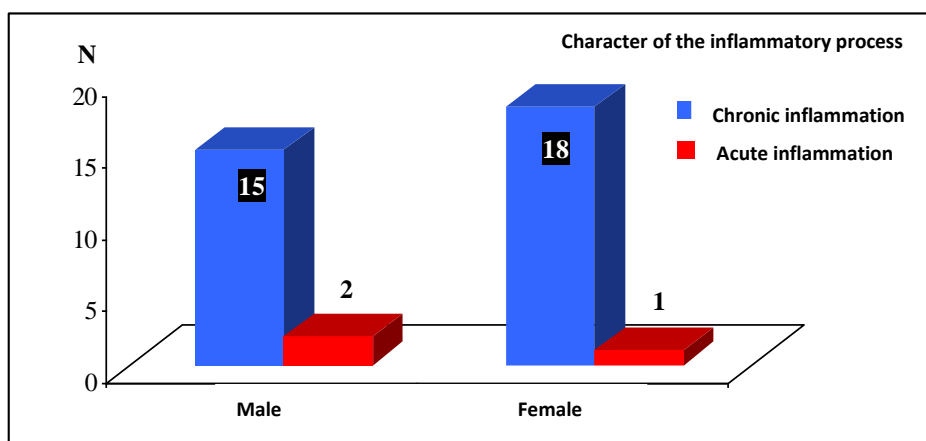


Figure 1. Character of the inflammatory process, p (Fisher exact) = 0.46 ns.

Surgical intervention lasted insignificantly longer in male patients ($p = 0.4$). The duration of the operative intervention ranged from 30 to 210 minutes, on average 69.1 ± 43.3 , while in female patients the duration of the intervention was from 20 to 150 minutes, on average 57.1 ± 28.4 minutes. In the group of male respondents the duration less than 50 minutes was confirmed in 7 (41%), from 51 to 100 minutes in 8 (47%) and longer than 100 minutes was verified in 2 (12%) respondents. In the group of female respondents duration less than 50 minutes was confirmed in 10 (53%), from 51 to 100 minutes in 8 (42%) and longer than 100 minutes was verified in 1 (5%) respondent. (Table 2)

The results from the table 2 showed that the sex of the respondents did not have a significant impact on the length of hospitalization ($p = 0.16$).

Male patients stayed in the hospital insignificantly longer (7.8 ± 8.3 vs. 5.6 ± 4.25). In the group of male respondents, hospital treatment shorter than 6 days was confirmed in 11 patients (65%), from 7 to 12 days in 3 (17.5%) and longer than 12 days of hospital treatment was confirmed in 3 (17.5%) respondents. The female group of respondents is characterized by a duration of less than 6 days in 15 (79%), from 7 days to 12 days in 3 (16%), and more than 12 days in 1 (5%) patient.

Table 2. Descriptive statistics on the duration of the surgical operation and days of hospitalization in both sexes.

Sex of the respondents	Descriptive statistics		p value
	mean ± SD	interval (min)	
Duration of the operative intervention			
Male	69.12 ± 43,27	30 – 210	^a p= 0.412ns
Female	57.11 ± 28,40	20 – 150	
Duration of hospital stay in days			
Male	7.76 ± 8,34	4 – 37	^b p=0.16 ns
Female	5.58 ± 4,25	3 – 22	

^a p (Mann-Whitney) , ^b p (Student's test)

Regarding complications in the operative and early postoperative course (obstructive jaundice, stenosis of the pancreatic part of d.choledochus, stenosis of the post-pyloric part of the duodenum, pericholecystic and hepatic abscesses, prolonged febrile condition, biliary peritonitis, partial cholecystectomy with adhesive block, cholecystoduodenal fistula) it turned out that in the total number of respondents (n = 36) 3 patients or 8.3% had one complication each, 2 patients 5.6% two complications each and more than three complications were verified in one patient, while in 30 patients or 83.3% no complications were noted.

Regarding gender correlation, insignificantly more frequent complications were noted in male patients - 5 (29%) vs.1 (5%), $p = 0.08$ (Fisher exact test). In the male group of subjects with one complication were 2 patients, one of whom had prolonged febricity, and the other with partial cholecystectomy with adhesive block, two patients were noted with two complications each, of whom one was with prolonged febrile, and the other with partial cholecystectomy with adhesive block, two patients were noted with two complications, one of whom was diagnosed with obstructive jaundice and stenosis of the pancreatic part of d. choledochus, and in the other patient with obstructive jaundice and stenosis of the postpyloric part of the duodenum.

In one patient 3 complications were noted (obstructive jaundice, biliary peritonitis, pericholecystic and hepatic abscesses). In the female group of patients, only one subject was diagnosed with a complication (cholecystoduodenal fistula).

Histopathological analysis of 36 gallbladder preparations revealed 12 hyperplasia (33%), 8 hyperplasia with erosion (22%), 1 hyperplasia with ulceration (3%), 13 erosions (36%) and 2 ulcerations (6%) of epithelium.

Gender difference in the histopathological finding, i.e. in the intensity of the inflammatory changes of the gallbladder epithelium was not confirmed ($p = 0.05$). The most common finding in male patients was epithelial erosion - 7 (41%), and in female hyperplasia - 7 (37%).Table 3.

Histopathological analyzes of gallbladder preparations in terms of inflammatory infiltrate density showed a quantitative presence of scanty infiltrate in 24 (67%) subjects, moderate infiltrate in 10 (28%) and abundant infiltrate in 2 (5%) subjects, (Table 3) and no static significant difference between male and female patients ($p = 1.0$)

Regarding the type of cells present in the inflammatory tissue infiltrate, as shown in table 3 in the total number of subjects the isolated presence of only lymphocyte cells was noted in 22 subjects (61%), lymphocytes with polymorphonuclear cells (PMN) in 12 (33%) and the presence of only polymorphonuclear inflammatory cells in 2 (6%) patients.

In terms of sex, in the male group of respondents, isolated lymphocyte presence was noted in 9 (53%) respondents, lymphocytes with polymorphonuclear inflammatory cells in 6 (35%) and presence of inflammatory infiltrate with the absence of lymphocyte cells in 2 (12%) respondents. In contrast to the male target group, in 14 (73.7%) females isolated lymphocyte presence and presence of mixed inflammatory infiltrate in the composition of lymphocyte and polymorphonuclear cells were found in 5 (26.3%).

Isolated presence of only polymorphonuclear cells was not verified in any member of this target group. This resulted that female group have only lymphocytic infiltration in comparison with male group which is proved with significant difference ($p=0.01$) at statistical tests.

Regarding the degree of fibrous changes, in 21 (58%) patients changes were verified with scanty fibrosis, in 9 (25%) with moderate, and changes with strongly expressed fibrosis were confirmed in 6 (17%) patients.

The degree of fibrotic changes did not differ significantly in male and female patients ($p = 1.0$), ie scanty fibrosis was found in 9 (53%) male and 12 (63%) female patients, moderate fibrosis in 4 (23.5%) male and 5 (26%) female patients, and severe fibrotic changes in 4 (23.5%) male and 2 (11%) female patients (Table 3).

Table 3. Descriptive statistics of pathohistological findings on the gallbladder wall and character of inflammatory infiltrate in both sexes

Variable	Sex of the respondents		p value
	Male	Female	
	n (%)	n (%)	
Histopathological analysis			
Hyperplasia	5 (29.4)	7 (36.8)	^a p= 0.05 sig
Hyperplasia with erosion	3 (17.6)	5 (26.3)	
Hyperplasia with ulceration	0	1 (5.3)	
Erosion	7 (41.2)	6 (31.6)	
Ulceration	2 (11.8)	0	
The density of the inflammatory infiltrate			
+ (scanty infiltrate)	11 (64.7)	13 (68.4)	^a p= 0.59ns
++ (moderate infiltrate)	5 (29.4)	5 (26.3)	
+++ (abundant infiltrate)	1 (5.9)	1 (5.3)	
The type of cells present in the inflammatory tissue infiltrate			
Lymphocytes	9 (52.9)	14 (73.7)	^a p= 0.01 sig
Lymphocytes + PMN	6 (35.3)	5 (26.3)	
PMN	2 (11.8)	0	
The degree of fibrous changes in the tissue preparation			
+ (scanty fibrosis)	9 (56.2)	12 (63.2)	^a p= 0.2ns
++ (moderate fibrosis)	4 (23.5)	5 (26.3)	
+++ (severe fibrosis)	4 (23.5)	2 (10.5)	

p (Fisher exact test) ,

Laboratory tests showed a significant difference between the two tested groups in the measured values of progesterone with a significance of $p = 0.027$ in favor of the female population, and for testosterone and IgA in favor of the male population ($p = 0.001$, $p = 0.036$, respectively), while in other markers examined (estradiol CRP, interleukin 2, interleukin 6 and ALT, AST, ALP, GGT, IgG, IgM) no significant difference between the sexes was found. (Table 4)

Table 4. Descriptive statistics of laboratory values in both sexes

Variables	MALE	FEMALE	p-value
	mean/SD	mean/SD	
CRP	23,69 ± 37,28	34,09 ± 59,51	0.388 ns
ESTRADIOL	75.23 ± 50.41	87.38 ± 75.96	0.199 ns
PROGESTERONE	0.29 ± 0.13	0.83 ± 1.75	0.027 sig
TESTOSTERONE	273.82 ± 145.9	23.95 ± 8.24	0.0001 sig
IL2	740.29 ± 621.33	614.84 ± 314.28	0.156 ns
IL6	17.83 ± 35.51	37.88 ± 95.49	0.134 ns
ALT	47.12 ± 30.95	39.74 ± 23.82	0.693 ns
AST	36.88 ± 24.02	30.32 ± 19.17	0.642 ns
ALP	100.53 ± 58.97	116.53 ± 122.42	0.544 ns
GGT	61.94 ± 76.33	59.37 ± 93.96	0.668 ns
IgG	9.89 ± 2.17	10.68 ± 1.67	0.651 ns
IgA	12.03 ± 37.90	2.18 ± 0.74	0.036 sig
IgM	0.97 ± 0.71	1.28 ± 0.65	0.776 ns

t (Student's test) **p<0.05

Discussion

The comparative analysis of the research results obtained data that show insignificance in terms of the duration of the operative intervention and the duration of the hospital stay, but the percentage values related to these measurement parameters confirm the longer duration of the operative interventions and the hospital stay in the male group respondents compared to the female group included in the study.

Gender difference in the histopathological finding, i.e. in the intensity of the inflammatory changes of the gallbladder epithelium was not significantly confirmed.

The most common finding in male patients was epithelial erosion, and in female patients hyperplasia. The higher percentage of erosive and ulcerative changes of the epithelium in the male group of respondents is in favor of a more intense acute inflammatory.

In retrospective study made by Peter C Amber in period of January 2009 to December 2013 at Saint Remigius Hospital Opladen, were included 676 patients with pathological diagnosis of cholecystitis. From them 138 patient were with acute cholecystitis. In the study was analyzed only 138 patients which had laparoscopic cholecystectomy.

They were divided in male group with 69 participants and female group with 69 participants. The results of the study showed that in male group have significant higher inflammatory response with gangrenous and necrotic changes than female group (43/69 vs 21/69, p=0.002).

This lead to conclusion that male gender is independent factor for worse prognosis of the cholecystitis (p=0.018) and this make need for early operative treatment.

Histopathological analyzes of gallbladder preparations regarding the inflammatory infiltrate density showed a quantitative presence of scanty, moderate abundant infiltrate with no statistically significant difference between male and female patients.

Regarding the type of cells present in inflammatory tissue infiltrate, results were obtained that show a higher percentage of inflammatory cells characteristic of acute or chronic with acute exacerbation of inflammation, in favor of the male group of respondents.

The described differences between the two sexes were not sufficient to confirm statistically as significant. Other retrospective study made by Mehrdad Nikfarjam from University of Melbourne, Australia, where was included 386 patients with cholecystitis shows that acute form of inflammation is more represent in male group (47%) than female group.

Also they concluded that in male population have more aggressive inflammatory response with gangrenous cholecystitis in male (45%) than female (23%) [16].

The degree of fibrous tissue changes in a tissue preparation marked by moderate and strongly expressed fibrosis showed a higher percentage in the male group of respondents but they are without statistically significant difference between the two sexes.

In terms of gender, insignificantly more frequent complications were noted in male patients. The results confirmed the more frequent presence of complications (obstructive jaundice, stenosis of the pancreatic part of the d. choledochus, stenosis of the postpyloric part of the duodenum, pericholecystic and hepatic abscesses, prolonged febrile condition, biliary peritonitis, conversion) associated with inflammatory disorders of the gallbladder characteristic of the male group of respondents.

The National Data Base of Denmark shows that from the total number of laparoscopic cholecystectomies, 7,7% are converted in open approach.

This result are proved by Simon E Thesbjerg in his study where was analyzed 5.951 participants with cholecystitis. He concluded that acute cholecystitis is more represented in male group (14%) and also the male group have more conversion (13.3%) than female group (5,8%) [7].

Laboratory tests showed a significant difference between the two tested groups in the measured values of progesterone with significance in favor of the female population, and for testosterone and IgA in favor of the male population.

There are studies which approved the influence of testosterone in modelling of inflammatory process via activation of proinflammatory cytokines and anti-inflammatory cytokines [9,15].

Fehrenbacher J. C and the associated in period of 2006 to 2009 year in USA made retrospective study in which was analyzed the interstitial fluid from the gallbladder tissue in 78 patients. The results shows higher values of Il-6 and Il-8 in both genders with acute cholecystitis while values of Il-1b and Il-10 were higher only in the male group [10].

In our study regarding the other examined markers (estradiol CRP, interleukin 2, interleukin 6 and ALT, AST, ALP, GGT, IgG, IgM) no significant difference between the sexes was found. In retrospective study by Fahad Mahmood and associates from Queen Elizabeth Hospital Birmingham in UK, in period of 5months were included 600 patients which have cholecystectomy shows that the age of the patient, CRP level and NLR are in depended factors for worse pathologic findings in inflamed gallbladder [13].

Conclusion

From the analysis it can be concluded that despite the small number of respondents included in the study which doesn't meet the required quantum to obtain relevant significance in most analyzed parameters, the percentage domination of acute and chronic inflammatory changes characteristic of cholecystitis as inflammatory disease of the gallbladder prove the impact of gender difference on the intensity of the inflammatory response, which in turn is an additional challenge for a more extensive study in this area.

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