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Original article

CORRELATION OF SERUM LEVELS OF INFLAMMATORY CYTOKINES WITH SEVERE FORM OF CHOLECYSTITIS

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Abstract

Introduction: Cholecystitis is an inflammatory response of the body triggered by a number of mutually supporting biological mechanisms, which by creating and releasing inflammatory mediators - cytokines activate the innate or acquired immune system, which leads to neutralization of the harmful stimulus and initiation of the process of repair and regeneration of damaged tissue or its continuation as a long-term chronic process with simultaneous tissue destruction and reparation.

Material and methods: The study was conducted at the General City Hospital (GCH) "8th September" in Skopje and the Institute of Immunology and Human Genetics in Skopje, in the period of 2020-2022. Statistical analysis of the data was performed with the statistical package SPSS for Windows 26.0.

Results: The study included 165 subjects with gallbladder inflammation divided into 3 groups: mild, moderate and severe inflammation grade. Patients with mild, moderate, and severe inflammatory processes differed significantly in IgG levels ($p = 0.049$), IgA ($p = 0.021$), and IgM ($p = 0.016$) and insignificantly in IgE1 levels ($p = 0.16$). Patients with a severe inflammatory process had a higher prevalence of IL-2R and IL-8 than patients with a mild grade ($p = 0.035$; $p = 0.26$, respectively). The intensity of inflammatory process had a non-significant effect on the levels of TNF-alpha ($p = 0.078$), and a significant effect on the levels of fibrinogen ($p = 0.001$), with significantly higher levels of fibrinogen in the group of patients with severe inflammatory process compared to the group with mild grade ($p = 0.0009$).

Conclusion: The intensity of inflammatory process affects the serum levels of inflammatory cytokines with presence of strong correlation between the severe form of cholecystitis and elevated serum levels of certain inflammatory cytokines.

Keywords: cholecystitis, inflammation, cytokines, interleukins, immunoglobulins

Introduction

Cholecystitis is an inflammatory disease of the gallbladder, and numerous general practitioners and surgeons have been dealing with this problem since 1420, starting with a primitive and ineffective symptomatic treatment until the first successful cholecystectomy was performed in 1867[1].

Although there are numerous predisposing etiological factors for the occurrence of cholecystitis, in 90 to 95% of cases the cause is calculosis[2,3].

Numerous pathological conditions, metabolic products and hormones affect the course and development of this inflammatory disease, the surgical treatment of which occupies a large percentage in abdominal surgery[4-6].

Cholecystitis is a reactive inflammatory response of the body triggered by a number of mutually supporting biological mechanisms, which by creating and releasing inflammatory mediators - cytokines (tumor necrotizing factor, interleukin 1,2,6,8, fibrinogen) activate the innate or acquired immune system (IgG, IgA, IgM and IgE1) that leads to neutralization of the harmful stimulus and initiation of the process of repair and regeneration of damaged tissue or its continuation as a long-term chronic process with simultaneous tissue destruction and reparations[7-15].

Cholecystitis as a defense inflammatory reaction is recognized in symptomatic and asymptomatic form with or without complications. The consequences of cholecystitis can range from mild inflammatory changes (acute and chronic) with mild histopathological changes in the gallbladder wall (edema, erosion, hyperplasia, mild fibrosis) to severe inflammatory processes with extremely severe histopathological changes which in the form of acute progressive (hydrops, empyema, gangrene, perforation) or chronic cholecystitis can produce a number of complications such as cholangitis, choledocholithiasis, jaundice, pancreatitis, fistula between gallbladder with biliary duct or organ (ductus choledochus, duodenum, small intestine and stomach), ileus, cancer, and lethal outcome[16-19].

Multiple etiological factors as well as the unpredictable course of inflammation are the reason for numerous scientific researches and studies which result is the creation of diagnostic criteria for assessing severity such as Tokyo Guidelines 2018: diagnostic criteria and severity grading of acute cholecystitis, in order to make a correct diagnosis, classification and prognosis of the severity of inflammatory process and taking appropriate therapeutic measures[2-24].

Different intensity of inflammatory process and thus a difference in the degree of organic damage is the reason for using standard criteria in pathological surgery, precisely defined in pathological surgery literature (such as *Robbins and Cotran: Pathological basis of disease, 8th edition by Vinay Kumar*), which in accordance with the pathohistological changes of the gallbladder make a classification of severity and intensity of inflammatory response in cholecystitis[17-19].

Materials and methods

The study was conducted at the GCH "8th September" in the period of 2020-2022, at the departments of abdominal surgery, pathology, laboratory and biochemical analysis, transfusiology and at the Institute of Immunology and Human Genetics at the Faculty of Medicine in Skopje.

Laboratory tests were performed on preoperatively taken blood samples and pathohistological analysis of a tissue sample on a surgically removed gallbladder.

In terms of design, the research is a prospective intervention study.

The study comprised 165 patients with gallbladder inflammation, treated as emergency or elective cases with laparoscopic approach. According to the intensity of inflammatory process, patients were divided into 3 groups: with mild, moderate and severe inflammation grade.

Inclusion criteria: patients operated as emergency and elective cases diagnosed with acute calculous and acalculous cholecystitis and patients aged 18 to 80 years.

Exclusion criteria: patients with other gallbladder pathology (malignancies, congenital biliary tract abnormalities) and comorbidities, who were contraindicated for surgical treatment.

The study data were provided by disease histories, operative protocols, and the results obtained from laboratory and pathohistological analyses.

The severity classification of cholecystitis was based on definitions described in ***Robbins and Cotran: Pathological basis of disease****, 8th edition by Vinay Kumar et al*., and in accordance with pathohistological changes of the gallbladder wall, as well as the **Tokyo guidelines for grading the severity of cholecystitis** (TG 18).

Based on the criteria described in the histopathological **Sakuramoto classification**[18], the grade of inflammatory response in correlation with the histopathological finding was divided into:

Acute cholecystitis

None:	Free of acute findings.
Slight:	Only inflammatory cell invasion, such as slight neutrophil infiltration.
Moderate:	Inflammatory cell invasions, such as moderate neutrophil infiltration edema of mucosal layer, epithelaxia, and erosion formation.
Severe:	Inflammatory cell invasions, such as severe neutrophil infiltration, visible abscess formation, hyperemia, bleeding, and mucosal ulceration.

Chronic cholecystitis

None:	Free of chronic findings.
Slight:	Lymph follicle formation and slight chronic inflammatory cell invasion.
Moderate:	Lymph follicle formation, chronic inflammatory cell invasion, and fibrosis to muscular layer or subserosal layer.
Severe:	Fibrosis in complete layers and destruction of the mucosal layer.

Statistical analysis of the data was performed with the statistical package SPSS for Windows 26.0. Kolmogorov-Smirnov test for normality and Shapiro-Wilk's W test were used to test the data normality.

Continuous variables are represented by mean ± SD or median (IQR), categorical variables with absolute numbers (%), i.e., with frequency distributions.

Comparison of the groups with different intensity of inflammatory process in relation to categorical variables was made with the Chi-square test, and the comparison in relation to continuous variables was performed with the Analysis of Variance, post-hoc Tukey honest or with Kruskal-Wallis, post-hoc Mann-Whitney test, depending on data distribution.

Levels of $p < 0.05$ were considered as statistically significant.

Pathohistological analysis was performed on the principle of submitted surgical tissue material - gallbladder with samples taken from previously macroscopically assessed zones with pathological changes. Data processing was performed by an automated process in a tissue processor Thermo Fisher Scientific Citadel 2000. The sections were analyzed under a light microscope Nicon Eclipse E600.

Biochemical analyses were performed using Siemens apparatus, Dimension RXL biochemical analyzer, by photometric method.

Results

The study included 165 patients with inflammation of the gallbladder, patients from PHI GCH "8th September", operated as emergency or elective cases.

According to the intensity of inflammatory process, patients were divided into 3 groups: with mild, moderate and severe inflammation grade (Figure 1).

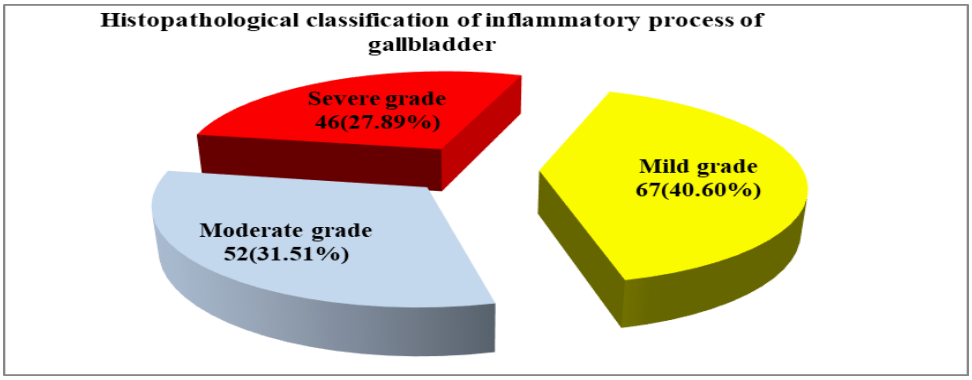

Fig. 1. Intensity of inflammatory process according to histopathological classification

The sex of patients had a significant effect on the intensity of inflammatory process (p = 0.0016). A more severe form of inflammatory process was registered in male patients, i.e., in the group of male patients the inflammatory process was most often manifested in a severe form (41.33%), while in the group of female patients most often as a mild form (48.89%).

The intensity of inflammatory process significantly depended on the age of patients (p = 0.000027). Patients with a severe form were on average the oldest (59.0 ± 12.6), followed by patients with moderate (52.4 ± 12.7) and mild inflammation (47.3 ± 13.1). The difference in the mean age between patients with a severe form *versus* patients with a mild form (p = 0.00003) and patients with a moderate grade of inflammatory process (p = 0.032) was confirmed as statistically significant (Table 1).

Table 1. Age, duration of surgery intervention and number of hospital days

Variable	Total	Grade of inflammatory process			p-level
		Mild n=67	Moderate n=52	Severe n=46	
Sex					
Male	75(45.45)	23(30.67)	21(28)	31(41.33)	X^2=12.8 **p=0.0016
Female	90(54.55)	44(48.89)	31(34.44)	15(16.67)	
Age					
mean± SD	52.18 ± 13.6	47.3 ± 13.1	52.4 ± 12.7	59.0 ± 12.6	F=11.2 ***p=0.000027
min – max	21 - 77	21 - 75	28 - 76	28 - 77	[b]p=0.00003,[c]p=0.032
Total number of hospitalization days					
mean± SD	4.1 ± 2.6	3.6 ± 1.6	3.6 ± 1.2	5.5 ± 4.1	H=14.85 ***p=0.0006
min – max	2 - 23	2 - 10	2 - 8	2 - 23	[b]p=0.0011,[c]p=0.021
median (IQR)	3(3-5)	3(3-4)	3(3-4)	4(3-6)	
Number of postoperative hospitalization days					
mean± SD	2.7 ± 1.9	2.4 ± 1.5	2.4 ± 0.9	3.8 ± 2.9	H=15.6 ***p=0.0004
min – max	1 - 15	1 - 9	1 - 6	1 - 15	[b]p=0.00083
median (IQR)	2(2-3)	2(2-3)	2 (2-3)	3(2-4)	
Duration of surgery intervention in minutes					
mean± SD	60.69 ± 27.8	53.66 ± 25.6	51.73 ± 20.7	81.09 ± 27.7	F=21.6 ***p=0.000000
min – max	20 - 205	20 - 205	20 - 105	35 - 165	[b]p=0.00002,[c]p=0.00002
Conversion from laparoscopic to open approach					
Yes	3(1.82)	0	0	3(6.52)	
No	154(93.33)	65(97.01)	51(98.08)	38(82.61)	
Open approach	8(4.85)	2(2.99)	1(1.92)	5(10.87)	

X^2 (Chi-square); F (Analysis of Variance, post –hoc Tukey honest); H (Kruskal-Wallis, post-hoc Mann-Whitney), [a]p (mild *vs*. moderate), [b]p(mild vs severe), [c]p (moderate *vs*. severe), **p<0.01; ***p<0.000

Total hospitalization and postoperative hospitalization differed significantly depending on the grade of the inflammatory process (p = 0.0006 and p = 0.0004, respectively). Table 1 illustrates

that patients with a severe grade had a significantly longer overall hospitalization than patients with mild (p = 0.0011) and moderate grade (p = 0.021). The average total number of hospital days was 3.6 ± 1.6, 3.6 ± 1.2 and 5.5. 4.1, respectively, in the groups with mild, moderate and severe inflammatory process. Postoperative hospitalization was significantly longer in patients with a severe grade than in patients with mild (p = 0.0004). The average number of hospital days was 2.4 ± 1.5, 2.4 ± 0.9 and 3.8. 2.9, respectively, in the groups with mild, moderate and severe inflammatory process.

The intensity of inflammatory process had a significant impact on the duration of surgery (p <0.0001). The intervention lasted significantly longer in patients with severe inflammation compared to patients with mild (81.09 ± 27.7 *vs.* 53.66 ± 25.6, p = 0.00002), and with moderate form (81.09 ± 27.7 *vs.* 51.73 ± 20.7, p = 0.00002).

Table 2 shows the results which prove a significantly higher prevalence of C-reactive proteins (p = 0.0024) in the severe form of cholecystitis compared to the mild ^{b}p (mild *vs.* severe), and moderate form, ^{c}p (moderate *vs*. severe) mean ± SD mild form (4.84 ± 4.0), moderate form (8.84 ± 15.9) and severe form (27.74 ± 54.2).

Table 2. Difference in serum CRP level in mild, moderate, and severe form of cholecystitis

Variable	n	Grade of inflammatory process			p-level
		Mild n=67	Moderate n=52	Severe n=46	
CRP (mg/L)					
mean ± SD		4.84 ± 4.0	8.84 ± 15.9	27.74 ± 54.2	H=12.02 p=0.0024
min – max		0.6 - 23.2	0.2 - 90.9	0.2 - 201	^{b}p=0.0111 ^{c}p=0.00534
median (IQR)		3.14(2.86 - 4.95)	2.96(2.86 - 5.55)	5.87(3.14 - 17)	X^2=11.08
< 5 n (%)	111	51(76.12)	38(73.08)	22(47.83)	p=0.0039
>5 n (%)	54	16(23.88)	14(26.92)	24(52.17)	^{b}p=0.002, ^{c}p=0.01

H (Kruskal-Wallis test); post-hoc Mann-Whitney, X^2 (Pearson Chi-square), ^{b}p (mild vs severe), ^{c}p (moderate vs severe)

Table 3. Difference in serum levels of IgG, IgA, IgM and IgE1 in mild, moderate and severe form of cholecystitis

Variable	n	Grade of inflammatory process			p-level
		Mild n=67	Moderate n=52	Severe n=46	
IgG (g/L)					
mean ± SD		11.46 ± 2.4	10.51 ± 2.33	10.49 ± 2.7	F=3.06 *p=0.049
min – max		6.97 - 21.4	3.14 - 16.2	5.04 - 17	^{b}p=0.049
IgA (g/L)					
mean ± SD		2.26 ± 0.7	2.23 ± 1.1	3.23 ± 3.3	H=7.76 *p=0.021
median (IQR)		2.19(1.75 - 2.86)	2.03(1.48 - 2.58)	2.52(1.95 - 3.45)	^{c}p=0.02
IgM (g/L)					
mean ± SD		2.99 ± 15.5	1.02 ± 0.8	0.96 ± 0.9	H=8.28 *p=0.016
median (IQR)		1.09(0.71 - 1.46)	0.86(0.52 - 1.22)	0.63(0.44 - 1.18)	^{b}p=0.017
IgE1 (U/mL)					
mean ± SD		91.15 ± 213.2	68.62 ± 101.4	130.36 ± 245.1	H=3.7 p=0.16
median (IQR)		19.8(17.1 - 44.9)	26.35(17.1 - 67.3)	39.55(17.1 - 119)	

F (Analysis of Variance, post –hoc Tukey honest); H (Kruskal-Wallis, post-hoc Mann-Whitney), ^{a}p (mild *vs.* moderate), ^{b}p (mild *vs.* severe), ^{c}p (moderate *vs*. severe), *p<0.05

The results presented in Table 3 show that patients with mild, moderate, and severe inflammatory processes differed significantly in levels of IgG (p = 0.049), IgA (p = 0.021), and IgM (p = 0.016), and insignificantly in IgE1 (p = 0.16).

Post-hoc analyses for intergroup comparisons showed that patients with mild inflammatory process had significantly higher IgG levels than patients with severe grade (mean = 11.46 ± 2.4 g / L *vs.* 10.49 ± 2.7 g/L, p = 0.049); patients with severe grade had significantly higher IgA levels than patients with moderate grade (median = 2.52 g / L *vs.* 2.03 g/L, p = 0.02); patients with mild grade had significantly higher IgM levels than patients with severe inflammatory gallbladder (median = 1.09 g/L *vs.* 0.63 g/L, p = 0.017).

Figures 2, 3, 4 and 5 show the distribution of patients with mild, moderate and severe grade of gallbladder inflammation, in relation to the distribution of IgG, IgA, IgM and IgE1 levels, analyzed as normal levels and levels that deviated from the reference ones.

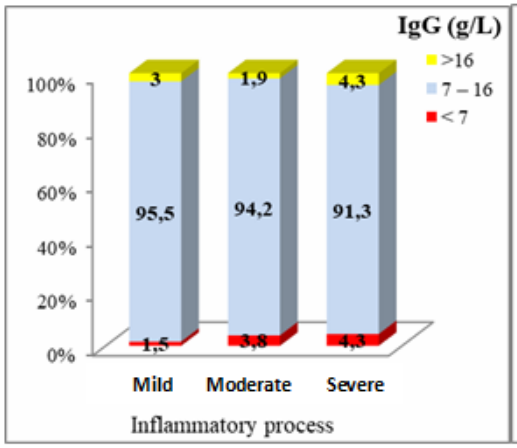

Fig. 2. Serum levels of IgG

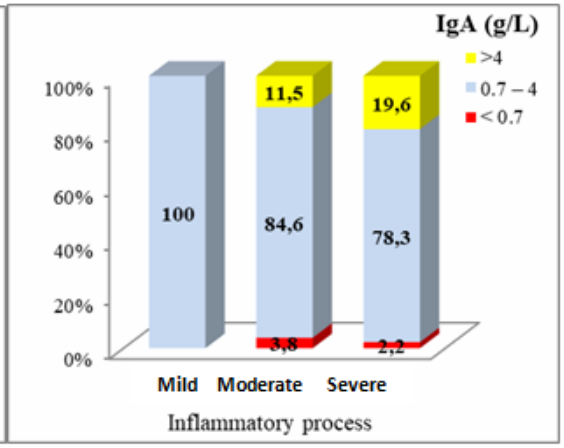

Fig. 3. Serum levels IgA

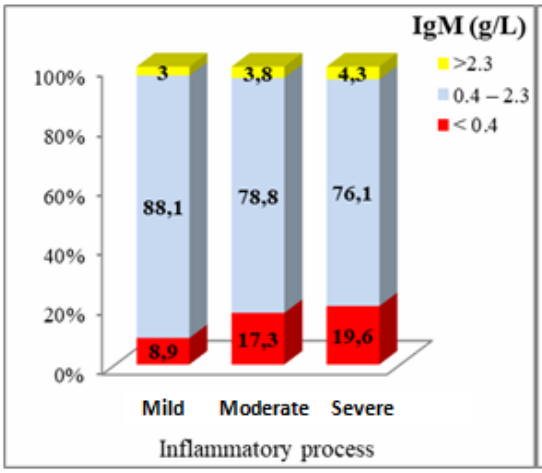

Fig. 4. Serum levels of IgM

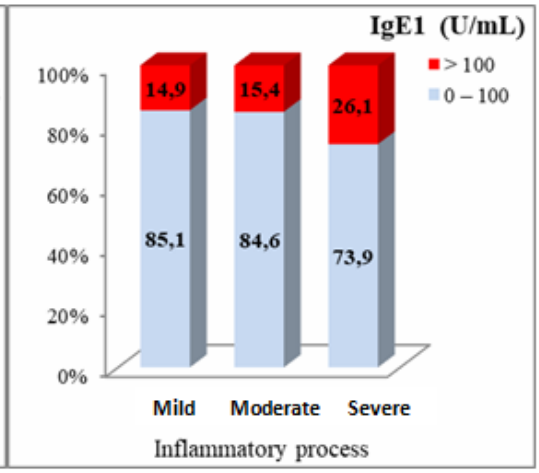

Fig. 5. Serum levels of IgE1

Table 4 shows a comparison of patients with mild, moderate, and severe inflammatory processes with respect to interleukins IL-1 beta, IL-2R, and IL-8, proving a statistically significant difference between groups in terms of IL- 2R and IL-8 (p = 0.035 and p = 0.024, respectively), and at the limit of significance with respect to IL-1 beta levels (p = 0.051).

Post-hoc analyses for intergroup comparisons showed that patients with a severe inflammatory process had significantly higher IL-2R and IL-8 levels than patients with mild (median = 506.5 U/ml *vs.* 403 U/ml, p = 0.035; median = 8.69 U/ml *vs.* 6.43 U/ml, p = 0.26, respectively).

Table 4. Serum levels of IL-1 beta, IL-2R and IL-8 in mild, moderate and severe form of cholecystitis

Variable	Grade of inflammatory process			p-level
	n	Mild n=67	Moderate n=52	Severe n=46	
IL-1 beta *(pg/ml)*				
mean ± SD		1.94 ± 0.1	1.91 ± 0.0	1.85 ± 0.2	F=3.06 p=0.051
min – max		1.91 - 2.61	1.91 - 1.91	0.98 - 1.91	
IL-2R *(U/ml)*				
mean ± SD		460.34 ± 191.5	521.77 ± 211.8	657.54 ± 411.8	H=6.7 *p=0.035
median (IQR)		403(348 - 547)	479(366 - 624)	506.5(360 - 829)	[b]p=0.035
IL-8 *(pg/ml)*				
mean ± SD		8.07 ± 7.5	13.72 ± 29.4	13.82 ± 24.1	H=7.47 *p=0.024
median (IQR)		6.43(4.18 - 9.25)	7.63(6.12 - 11.33)	8.69(6.57 - 11.83)	[b]p=0.026

F (Analysis of Variance, post –hoc Tukey honest); H (Kruskal-Wallis, post-hoc Mann-Whitney), [b]p (mild *vs.* severe), *p<0.05

Figures 6 and 7 show the distribution of patients with mild, moderate, and severe gallbladder inflammation in relation to the distribution of IL-2R, and IL-8 levels, analyzed as normal levels that deviated from the reference ones.

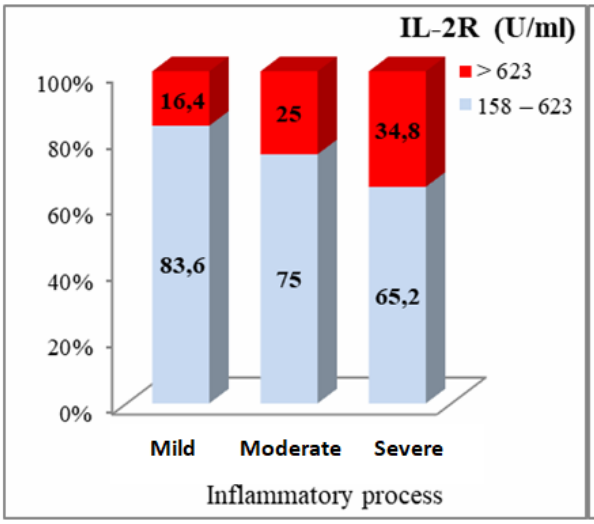

Fig. 6. Serum levels of IL-2R

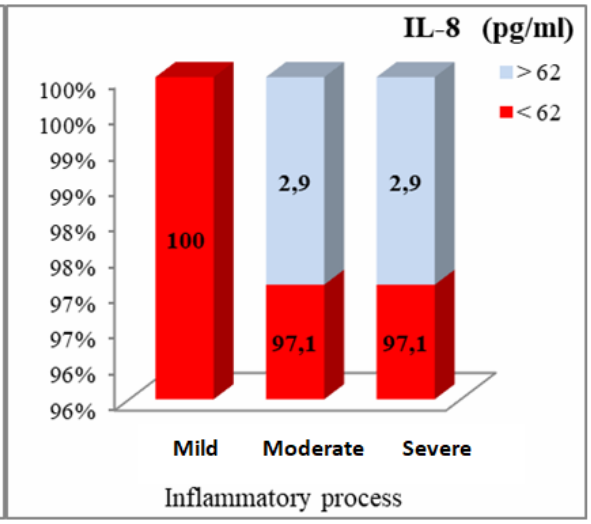

Fig. 7. Serum levels of IL-8

The intensity of inflammatory process had a non-significant effect on the levels of TNF-alpha parameter (p = 0.078), and a significant effect on the levels of fibrinogen (p = 0.001). Post-hoc analysis for intergroup comparisons showed that this overall significance was due to significantly higher levels of fibrinogen in the group of patients with a severe inflammatory process compared to the group with a mild grade (median = 3.15 g/l *vs.* 2.3 g / l, p = 0.0009), as shown in Table 5.

Table 5. Serum levels of TNF-alpha and fibrinogen in mild, moderate and severe form of cholecystitis

Variable	Grade of inflammatory process			p-level
	n	Mild n=41	Moderate n=34	Severe n=34	
TNF - alpha *(pg/ml)*				
mean ± SD		1.40 ±0.7	1.63 ± 1.1	1.85 ± 0.8	H=5.1 p=0.078
median (IQR)		1.39(0.93 - 1.86)	1.63(0.93 - 2.04)	1.86(1.39 - 2.44)	
Fibrinogen *(g/l)*				
mean ± SD		2.67 ± 1.4	3.20 ± 1.7	3.63 ± 1.9	H=13.8 **p=0.001
median (IQR)		2.3(2.1 - 2.9)	2.8(2.2 - 3.9)	3.15(2.5 - 4.5)	[b]p=0.0009

H (Kruskal-Wallis, post-hoc Mann-Whitney), [b]p (mild *vs.* severe), **p<0.01

Figure 8 shows the distribution of patients with mild, moderate, and severe gallbladder inflammation in terms of the distribution of TNF-alpha and fibrinogen levels, analyzed as normal levels, and levels that deviated from the reference ones.

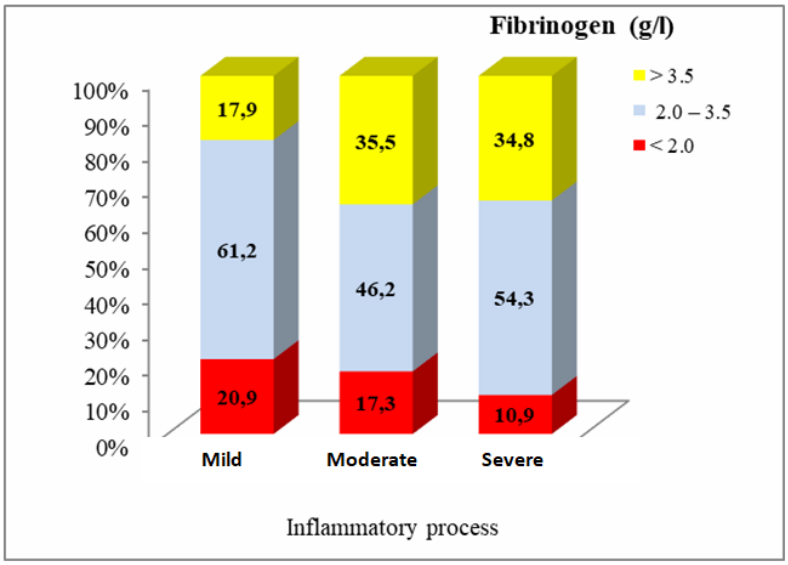

Fig. 8. Serum levels of fibrinogen

Discussion

The results obtained in our study showed that sex and age of patients had a significant effect on the intensity of inflammatory process ($p = 0.0016$ and $p = 0.000027$). A more severe form of inflammatory process was registered in male patients, i.e., in the group of male patients the inflammatory process was most often manifested in a severe form (41.33%), while in the group of female patients most often as a mild form (48.89%).

Patients with severe form were on average the oldest (59.0 ± 12.6), followed by patients with moderate (52.4 ± 12.7) and mild inflammation (47.3 ± 13.1). The difference in the mean age between patients with severe form *versus* patients with mild ($p = 0.00003$) and patients with moderate grade of inflammatory process ($p = 0.032$) was confirmed as statistically significant.

A retrospective study was performed at Monte Sinai Hospital in Juiz de Fora, Minas Gerais (Brazil) in the period between 2012 and 2016, which included 95 patients undergoing laparoscopic cholecystectomy with a mean age of 59.5 ± 17.1 years (mean age, 61.0 years). Only male patients with a clinical and pathological diagnosis of acute cholecystitis were included in the study. The results of multivariate logistics evaluation showed that approximately one third (29.3%, $n = 27$) of patients were diagnosed with gangrenous form of cholecystitis with GC, and all patients were older than 50 years[23].

J.U. Chong, J. H. Lee, Y. C.Yoon *et al.* in the period from August 1, 2012 to August 31, 2012, conducted a retrospective study which included a total of 8 different hospitals (Severance Hospital, Gangnam Severance Hospital, Wonju Severance Christian Hospital, Incheon & Daejeon St Mary Hospital, National Health Insurance Service Ilsan Hospital, Seoul National University, Bundang Hospital, and Guro Hospital) in South Korea in order to identify the factors that affect the length of postoperative hospital stay (PHS) after a performed laparoscopic cholecystectomy. The study included a total of 336 patients ($n = 336$) who underwent laparoscopic cholecystectomy for benign gallbladder disease and were divided into two groups depending on the length of PHS, namely the group for early discharge with 2 days or less and the group for late release with more than 2 days of postoperative stay. The postoperative hospital day was defined as the number of days from the day of surgery (day 0)

till discharge from the hospital. The average postoperative day was 2 days (range: 0-18 days). The average age of patients was 52 years (range: 14-85 years), of which 141 (42%) were men and 195 (58%) women. Factors that influenced PHS were divided into perioperative factors and factors that were related on the respondent-patient. Perioperative factors included time of surgery, perioperative transfusion, emergency surgery, previous history of abdominal surgery, acute gallbladder inflammation defined by histopathological examination, gallstones, and surgical site infection. The mean time of operative intervention was 45 minutes in the group with short hospital stay and 77 minutes in the group with long hospital stay, with defined significance ($p < 0.001$). There were 20.7% of respondents from the group with longer hospital stay that were operated as emergencies compared to 4.0% of respondents from the group with short hospital stay, with a significance of $p < 0.001$. A significant difference ($p < 0.001$) was also identified in the character of the inflammatory process, i.e., acute inflammation was more common in the group of respondents with a longer hospital stay. Regarding the age of patients, the study showed that the group with longer hospital stay was dominated by patients over the age of 65, and that was 75 (67.6%) *versus* 36 (32.6%). The sex of patients did not significantly affect the group of respondents with a longer hospital stay.The results in the study confirmed that the age of patients, prolonged time of surgical intervention and the nature of inflammatory process (acute cholecystitis) were independent factors that contributed to prolonged hospital stay [22].

In our study, total hospitalization and postoperative hospitalization differed significantly depending on the grade of the inflammatory process ($p = 0.0006$ and $p = 0.0004$, respectively). Patients with a severe grade had a significantly longer overall hospitalization than patients with mild ($p = 0.0011$) and moderate grade ($p = 0.021$). The average total number of hospital days was 3.6 ± 1.6, 3.6 ± 1.2 and $5.5. \pm 4.1$, respectively, in the groups with mild, moderate and severe inflammatory process. Postoperative hospitalization was significantly longer in patients with a severe grade than in patients with a mild grade ($p = 0.0004$). The average number of hospital days was 2.4 ± 1.5, 2.4 ± 0.9 and $3.8. \pm 2.9$, respectively, in the groups with mild, moderate and severe inflammatory process.

The intensity of inflammatory process had a significant impact on the duration of surgery ($p < 0.0001$). The intervention lasted significantly longer in patients with severe inflammation compared to patients with mild (81.09 ± 27.7 *vs.* 53.66 ± 25.6, $p = 0.00002$), and compared to patients with moderate form (81.09 ± 27.7 *vs.* 51.73 ± 20.7, $p = 0.00002$).

Our results proved a significantly higher prevalence of C-reactive proteins ($p = 0.0024$) in the severe form of cholecystitis compared to the mild ^{b}p (mild *vs*. severe), and moderate form, ^{c}p (moderate *vs*. severe), mean ± SD mild form (4.84 ± 4.0), moderate form (8.84 ± 15.9) and severe form (27.74 ± 54.2).

Similar to our research, in the period from 2015 to 2017 at the Hospital de Laredo (Cantabria, Spain) a retrospective study was conducted to prove the relation and predictive role of the C-reactive period in a severe form of cholecystitis. The study included a total of 115 patients who underwent surgery with a diagnosis of acute cholecystitis. According to pathological findings, patients were divided into two classification groups, of which 32 were diagnosed with gangrenous form of cholecystitis, and 83 with non-gangrenous form of cholecystitis. The results obtained in the study proved significantly higher levels of C-reactive protein in the group with gangrenous cholecysts ($p = 0.042$) and CRP ($p < 0.0001$), thus characterizing the C-reactive protein as a predictor factor for a severe form (gangrenous) of cholecystitis[23].

The results in our study confirmed that patients with mild, moderate, and severe inflammatory processes differed significantly in levels of IgG ($p = 0.049$), IgA ($p = 0.021$), and IgM ($p = 0.016$). Patients with a mild inflammatory process had significantly higher IgG levels than patients with severe (mean = 11.46 ± 2.4 g/L *vs.* 10.49 ± 2.7 g/L, $p = 0.049$), patients

with a severe grade had significantly higher IgA levels than patients with a moderate grade (median = 2.52 g/L *vs*. 2.03 g/L, p = 0.02), patients with a mild grade had significantly higher IgM levels than patients with a severe inflammatory gallbladder (median = 1.09 g/L *vs*. 0.63 g/L, p = 0.017).

Regarding the association of cytokines with a severe form of inflammatory process, our study demonstrated a statistically significant difference between groups in terms of IL-2R and IL-8 (p = 0.035 and p = 0.024, respectively), and at the limit of significance with respect to IL-1 beta levels (p = 0.051). Patients with severe inflammatory process had significantly higher IL-2R and IL-8 levels than patients with mild (median = 506.5 U/ml *vs*. 403 U/ml, p = 0.035; median = 8.69 U/ml *vs.* 6.43 U/ml, p = 0.26, respectively).

Z. Liu, T.J. Kemp, Y.T. Gao *et al.* conducted a study that measured the serum levels of a total of 13 cytokines including 10 interleukins associated with cancer and collected from a group of 150 patients diagnosed with gallbladder callus and a control group of 149 subjects. The study was conducted in the period between 1997 and 2001 in Shanghai, China. The results of the study showed a correlation between higher serum levels of interleukin (IL) IL-6, IL-10, IL-12 (p70) and IL-13 with an increased risk of cholecystolithiasis (i.e., p = <0.003, Bonferroni corrected). In line with previous research, the authors have proven that some cytokines (i.e., IL-1α, IL-6, IL-8, and tumor necrosis factor [TNF] -α) are associated with gallbladder disease. They showed a lower association of cytokines with the risk of cholecystolithiasis presence compared to the cytokine association and an increased risk of gallbladder cancer presence. The study concluded that four of the 13 circulating serum cytokines examined, were associated with cholecystolithiasis presence[25].

G. D. Buono *et al*. conducted a study that included a total of 223 patients who underwent laparoscopic cholecystectomy between January 2015 and December 2019 at the Department of General and Emergency Surgery of the University Hospital Policlinico of Palermo. The study was performed according to certain inclusion criteria and the subjects were divided into two groups, a group of 86 patients with severe laparoscopic cholecystectomy consisting of 45 male and 41 female subjects with a mean age of 65.51 ± 13.49, and a control group (mild form of laparoscopic cholecystectomy) consisting of 86 patients, 39 male and 47 female with a mean age of 55.47 ± 16.16 years. The aim of the study was to find predictive factors for severe laparoscopic cholecystectomy. The study analyzed a number of predictive factors, including the correlation of serum levels of fibrinogen with severe cholecystectomy. The results of the study confirmed a significant association of higher fibrinogen levels in the group of subjects with severe cholecystectomy compared to the control group of subjects, i.e., 466.95 U/L (SD: 210.19) *versus* 368.84 U/L (SD: 148.55), p value 0.006. The study demonstrated a significant association between elevated serum fibrinogen levels and severe laparoscopic cholecystectomy[22].

In our research, it was shown that the intensity of inflammatory process had a significant effect on the fibrinogen levels (p = 0.001). Post-hoc analysis for intergroup comparisons showed that this overall significance was due to significantly higher levels of fibrinogen in the group of patients with severe inflammatory process compared to the group with mild grade (median = 3.15 g/l *vs*. 2.3 g/l, p = 0.0009). The research found a non-significant effect on the levels of TNF-alpha parameter (p = 0.078).

Conclusion

The intensity of inflammatory process affects the serum levels of inflammatory cytokines with a presence of a strong correlation between the severe form of cholecystitis and elevated serum levels of certain inflammatory cytokines.

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