Gallbladder histological alterations in patients undergoing cholecystectomy for cholelithiasis.

Alterações histológicas da vesícula biliar de doentes submetidos à colecistectomia por colelitíase.

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A B S T R A C T

Objective: to describe the histological findings of the gallbladders of patients undergoing cholecystectomy and to evaluate the presence of factors associated with gallbladder incidental cancer. **Methods:** we conducted a descriptive, cross-sectional, observational study with 1,278 histopathological exams of gallbladders coming from cholecystectomy for cholelithiasis and of their reports, held from January 2008 to December 2017. **Results:** the most common pathological finding was chronic cholecystitis, present in 1,251 patients (97.8%), followed by gallbladder cholesterolosis, in 131 (10.2%). Gallbladder cancer was identified in six patients, with a prevalence of 0.5% in this sample. There was a significant association between the presence of cancer and age ≥60 years and wall thickness ≥0.3cm. **Conclusion:** there was low prevalence of gallbladder cancer in this population, higher occurrence in the elderly and association of the tumor with gallbladder wall thickness.

Keywords: Cholelithiasis. Cholecystectomy. Gallbladder Neoplasms.

INTRODUCTION

The high prevalence the gallstones in the population has made cholecystectomy one of the commonly conducted surgical procedures today. Anatomopathological studies of surgical specimens from cholelithiasis cholecystectomies, in some cases, uncover incidental gallbladder neoplasia, which in its initial phase is asymptomatic[1]. In the USA, 1-2% of patients submitted to cholecystectomy for cholelithiasis have gallbladder cancer at histopathological examination, and more than 80% of patients with gallbladder cancer have a prior history of cholelithiasis[2-5]. The evolution of the disease, however, is fast and has a high mortality rate.

The presence of stones and polyps, porcelain gallbladder, primary sclerosing cholangitis, chronic infection, congenital biliary cyst, obesity and diabetes are some of the risk factors for gallbladder cancer[6-8]. The increasing prevalence of the disease and the increase in life expectancy suggest that there should be an increase in the number of gallbladder cases in the coming years.

Given the association between historical findings of surgical specimens and development of malignancy[9-11], this work aims to describe the histological findings of the gallbladders of patients undergoing cholecystectomy and to evaluate the presence of factors associated with incidental cancer.

METHODS

We conducted a descriptive, individualized, cross-sectional, observational study, with 1,278 pathological examinations of requests coming from gallbladders cholecystectomy for cholelithiasis and their reports, in the period from January 2008 to December 2017. We selected the sample from a database of the pathology laboratory of the Lauro Wanderley University Hospital of the Federal University of Paraíba (HULW-UFPB).

For inclusion in the sample, patients' exams should include name, gender, age, pathological report, clinical data present the diagnosis of cholelithiasis as justification for cholecystectomy.

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We excluded from the sample the requests in which the hypothesis of gallbladder neoplasia was raised in the preoperative period.

The patients were operated by several surgeons of the same team. After removal of the gallbladder from the abdominal cavity, a macroscopic examination was performed by the surgeon and the specimen was then referred for anatomopathological examination in a 10% formaldehyde solution. In the Pathological Anatomy Service, the specimen was again submitted to macroscopic evaluation, and the suspected areas were properly treated and mounted on glass slides for microscopic analysis. In the absence of any suspicious areas, the specimen was subjected to routine examination, in which a random sample of the fundus, body and vesicular neck were analyzed.

The following histological changes were studied: chronic cholecystitis, acute cholecystitis, scleroatrophy, gangrene, abscess, xantogranulomatous cholecystitis, fibrosis, cholesterolosis, pyloric metaplasia, intestinal metaplasia, dysplasia and cancer.

We divided the patients into two groups as to age: under 60 years and 60 years or more. We classified the thickness of the gallbladder wall as thin (<0.3cm) or thick (≥0.3cm).

We performed descriptive analysis of the data and then we used the Pearson's chi-square test (x^2) to assess associations between histological alterations and gender, age range and gallbladder wall thickness. In cases where there was no possibility of applying the chi-square test, we replaced it by the Fisher's exact test. In all tests, the null hypothesis rejection level was set at 5%.

This research was approved by the Ethics in Research Committee of the Medical Sciences Center of the Federal University of Paraíba, with CAAE protocol number 01759418.5.0000.806 9.

RESULTS

Of the 1,278 reports under analysis, 992 (77.6%) were from females and 286 (22.4%), from males. The mean age was 43±17.8 years, 43±17 years for women and 44±20.6 years for men; 1,051 (82.2%) patients were under 60 years old and 227 (17.8%), 60 years or older. Of the 1,278 patients diagnosed with cholelithiasis, 1,261 (98.7%) were symptomatic before surgery, while only 17 (1.32%) had no symptoms.

Table 1 shows the frequency of the histological changes studied, regardless of the association between two or more diagnoses in the same patient, which occurred in some cases. The most common anatomopathological finding was chronic cholecystitis, which was present in 1,251 patients (97.8%), followed by cholesterolosis in 131 (10.2%). Gallbladder cancer was found in only six patients (0.5%). Figure 1 shows the histological aspects observed in some of the gallbladder histological changes.

Table 1. *Frequency distribution of histological changes.*

Histological change	no	%
Chronic cholecystitis	1,252	97.8
Cholesterolosis	131	10.2
Acute cholecystitis	36	2.8
Xantogranulomatous cholecystitis	23	1.8
Fibrosis	23	1.8
Scleratrophy	15	1.2
Dysplasia	13	1.0
Intestinal metaplasia	6	0.5
Cancer	6	0.5
Abscess	4	0.3
Pyloric metaplasia	2	0.2
Gangrene	1	0.1

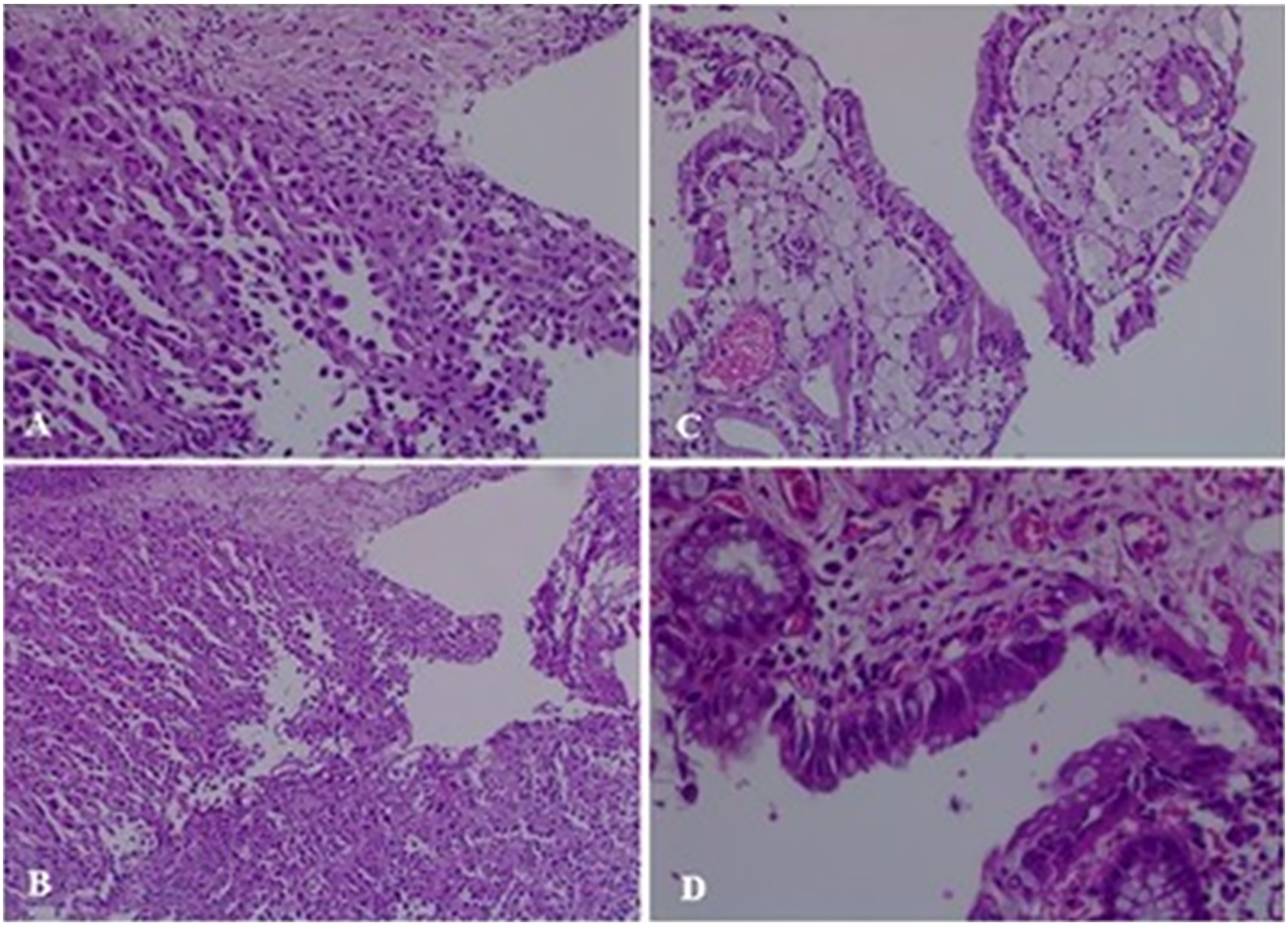

Figure 1. *Optical microscopy of the gallbladder and its histological changes: A and B) adenocarcinoma; C) cholesterolosis; D) low-grade epithelial dysplasia.*

Among patients diagnosed with cholelithiasis, 1,054 (82.4%) showed only one type of histological change, whilst the others had two (215; 16.8%) or three (9; 0.7%) concomitant changes. In patients with more than one concomitant histological change (224 patients), the most frequent association was between the chronic cholecystitis and cholesterolosis, representing 61.4% of the associations.

Tables 2 and 3 show the distributions of pathological findings by gender and age group (<60 years and ≥60 years), respectively. We observed statistically significant difference between the presence of cholesterolis, xanthogranulomatous cholecystitis and abscess in relation to the patients' gender.

Here was statistically significant difference between the presence of cholesterolosis, dysplasia and cancer in relation to patient age (<60 or ≥60 years).

Regarding wall thickness, 895 (70%) gallbladders had walls <0.3cm (thin), while in 383 (30%) the walls were ≥0.3cm (thick). Table 4 shows the distribution of histological changes in relation to wall thickness. There were statistically significant association between chronic cholecystitis, cholesterolosis, acute cholecystitis, Xanthogranulomatous cholecystitis, fibrosis, dysplasia, cancer, and abscesses in relation to the gallbladder wall thickness.

Table 2. *Distribution of histological changes in relation to gender.*

Histological change	Female		Male		p-value
	n	%	n	%	
Chronic cholecystitis	970	77.6	281	22.4	0.5537
Cholesterolosis	111	84.7	20	15.3	0.0399*
Acute cholecystitis	25	69.4	11	30.6	0.3153
Xantogranulomatous cholecystitis	13	56.5	10	43.5	0.0179*
Fibrosis	17	73.9	6	26.1	0.8056
Scleratrophy	10	66.7	5	33.3	0.3413
Dysplasia	7	53.8	6	46.2	0.0862
Intestinal metaplasia	5	83.3	1	16.7	1.0000
Cancer	6	100	0	0.0	0.3288
Abscess	0	0.0	4	100	0.0035*
Pyloric metaplasia	2	100.0	0	0.0	1.0000
Gangrene	0	0.0	1	100	0.2304

** p<0.05 (statistically significant difference).*

Table 3. *Frequency distribution of histological changes in relation to age.*

Histological change	<60		≥60		p-value
	n	%	n	%	
Chronic cholecystitis	1,030	82.4	221	17.6	0.39880
Cholesterolosis	120	91.6	11	8.4	0.00400*
Acute cholecystitis	25	69.4	11	30.6	0.05300
Xantogranulomatous cholecystitis	19	82.6	4	17.4	1.00000
Fibrosis	18	78.3	5	21.7	0.78210
Scleratrophy	13	86.7	2	13.3	0.75460
Dysplasia	5	38.5	8	61.5	0.00050*
Intestinal metaplasia	4	66.7	2	33.3	0.60070
Cancer	2	33.3	4	66.7	0.01350*
Abscess	2	50.0	2	50.0	0.155140
Pyloric metaplasia	1	50.0	1	50.0	0.30830
Gangrene	0	0.0	1	100.0	0.18190

** p<0.05 (statistically significant difference).*

Table 4. *Frequency distribution of histological changes in relation to wall thickness.*

Histological change	Thin		Thick		p-value
	n	%	n	%	
Chronic cholecystitis	889	71.1	362	28.9	0.0005*
Cholesterolosis	102	77.9	29	22.1	0.0005*
Acute cholecystitis	10	27.8	26	72.2	0.0489*
Xantogranulomatous cholecystitis	5	21.7	18	78.3	0.0005*
Fibrosis	11	47.8	12	52.2	0.0249*
Scleratrophy	12	80.0	3	20.0	0.4348
Dysplasia	5	38.5	8	61.5	0.0175*
Intestinal metaplasia	2	33.3	4	66.7	0.0799
Cancer	0	0.0	6	100.0	0.0005*
Abscess	0	0.0	4	100.0	0.0069*
Pyloric metaplasia	2	100.0	0	0.0	0.5632
Gangrene	0	0.0	1	100.0	0.2994

** p<0.05 (statistically significant difference).*

Of the 1,278 studied patients, six (0.5%) had incidental gallbladder cancer. In such patients, there was a range of ages between 54 and 74 years old, all were female, with symptoms of cholelithiasis prior to surgery, and thick-walled gallbladders. In two of the six patients, the neoplasm was associated with other histological changes, one with chronic cholecystitis and the other with dysplasia. We also found two cases of pyloric metaplasia, six cases of intestinal metaplasia and 13 cases of gallbladder dysplasia, all considered pre-neoplastic histological changes.

DISCUSSION

The gallbladder cancer is a rare malignancy, with aggressive character and low survival rates. Its largest incidences were reported in women in India (21.5/100,000), in Pakistan (1.8/100,000) and Ecuador (12.9/100,000)[12]. Several risk factors have already been associated with gallbladder neoplasia (GBN), such as obesity, multiparity, and chronic *Salmonella typhi* and *Helicobacter pylori* infection. However, the highest relative risk was associated with a cholelithiasis diagnosis, with relative risk (RR) of 4.9 (95%CI: 3.3-7.4), demonstrating that patients diagnosed with cholelithiasis are almost five times more likely to develop GBN[12-15].

According to Datasus data, from January 2008 to April 2019, cholelithiasis and the acute cholecystitis were responsible for more than 2.5 million hospitalizations in Brazil[8]. In this same period, the digestive tract surgeries reached the second place among the most performed surgical procedures, behind only the obstetric surgeries. Among the operations of the gastrointestinal tract, the most common is the cholecystectomy, with over 2 million procedures[16].

In the 1,278 gallbladders studied, we found 1,511 histopathological diagnoses, since 224 individuals presented more than one finding. Among these, chronic cholecystitis showed the highest prevalence, in 97.8% of patients, and was the most commonly associated with pyloric metaplasia, intestinal metaplasia and dysplasia. It is important to note that two of the six patients with intestinal metaplasia had associated dysplasia.

The second most found alteration was cholesterolosis, present in 10.2% of the patients. We observed a significantly higher occurrence of this alteration among females, individuals under 60 years old, and whose gallbladder had a thickness <0.3cm. Cholesterolosis is a non-inflammatory alteration of the gallbladder, having as pathophysiology the accumulation of lipids in the wall and the formation of cholesterol polyps, so far without known association with malignant transformation[17].

Xantogranulomatous cholecystitis (XC), an uncommon and destructive inflammation of the gallbladder, was the fourth most common alteration. We identified a statistical association between this change and wall thickness ≥0.3cm, as well as with female sex. Due to its ability to extend to adjacent structures, it can be confused with a neoplastic process. A study of more than 2,000 patients showed a positive association between XC and GBN[3].

The present sample is in line with other Brazilian series, such as the work of Oliveira e Silva *et al.*[10], which prospectively analyzed 290 patients undergoing laparoscopic cholecystectomy, finding chronic cholecystitis in 71.7% of patients, and acute inflammation, in 13.1%.

The treatment of GBN can range from a simple cholecystectomy, when the tumor is restricted to the mucosa, to the need for partial hepatectomy and resection of adjacent structures at more advanced stages[18].

The diagnosis and early treatment of symptomatic cholelithiasis are pointed as the main form of secondary prevention of this neoplasia[12,14], since numerous studies have already demonstrated the incidental occurrence of early-stage GBN in patients undergoing elective cholelithiasis[13,19].

In the present study, of the 1,278 patients, six (0.5%) had the diagnosis of gallbladder incidental neoplasia, and we observed that GBN was significantly more frequent among individuals aged 60 years or older, and in patients whose gallbladders had a wall thickness ≥0.3cm. The numbers found in this study are similar to data observed in other national and international studies. A cross-sectional study held in Pernambuco showed that in 2,018 evaluated patients there was a prevalence of 0.34% of cancer, the majority in females[12]. An European multicentric study a found prevalence of 0.35% among 117,840 patients[14], and in Buenos Aires, a survey found a prevalence of 0.91% of incidental GBN[20].

The occurrence of GBN in our sample is close to that found in the neighboring state, Pernambuco, reflecting a probable genetic similarity between these two populations and the importance of this factor in the origin of GBN. However, there is a substantial difference between these two states and the State of Maranhão, where the prevalence of incidental GBN is 2.3%. The incidence of GBN is known to be considerably lower in whites when compared with Asian, Hispanic and Black populations. Given that according to PNAD 2005, the proportion of whites in the population of Maranhão is only 25.7%, *versus* 36.1% in Paraíba and 37.4% in Pernambuco, this may be the explanation for the different prevalence found.

However, it is not possible to explain exactly why certain areas, such as India and Ecuador, have such a high incidence. It is evident that, despite the importance of environmental factors, genetic susceptibility still plays an important role in this neoplasia[14,21].

We should emphasize a significant aspect about the differences in the representativeness of histopathological findings in the gallbladder examination. Studies suggest that the incidence of various histological changes in the gallbladder, including GBN, is a reflection of the number of gallbladder segments analyzed. Limited analysis (a random sample of the fundus, body and vesicular neck) would not be sufficient to detect all cases of neoplasia. In contrast to the prevalence found in our series (0.5%), a study of 475 specimens analyzed throughout its length found a prevalence of 1.68% incidental GBN[13].

With regard to sex distribution, although all patients diagnosed with cancer in this sample are women, there was no statistically significant difference between these variables ($p>0.05$). However, it is well established in the literature that GBN is more common in females[12,15,19], which can be explained by the high prevalence of cholelithiasis in the female population as a result of hormonal factors that decrease the solubility of cholesterol in the bile, facilitating the formation of calculi.

The increased incidence of GBN in the elderly appears not to be directly related to age, but to the time course of cholelithiasis in these patients[9]. Studies on the carcinogenesis of GBN have shown that a history of at least 20 years of cholelithiasis is necessary for the onset of the first neoplastic changes[14]. There was also a progressive increase in the age of patients with preneoplastic changes.

The mean age of patients was 57.7 for intestinal metaplasia, 58.5 for pyloric metaplasia, 59.8 for dysplasia, and 64.2 for GBN. This finding reinforces the hypothesis of the sequence metaplasia-dysplasia-cancer in the carcinogenesis of GBN. The average ages of patients with dysplasia and carcinoma *in situ* were, respectively, 15 and five years younger compared with the average of patients with invasive cancer, suggesting that there was a temporal progression of these findings[14].

Due to the lack of information about the characteristics of gallstones in the analyzed requests, it was not possible to evaluate the relationship of these variables with the anatomopathological changes. Nevertheless, studies suggest an association between GBN and gallstones larger than 3cm. In this case, the risk of GBN is up to ten times higher compared with patients with stones smaller than 1cm[19]. GBN also seems to be more common in patients with a single, large stone[21].

In addition to the association with age, we also observed a statistically significant relationship between GBN and wall thickness greater than or equal to 0.3cm, which reflects the process of gallbladder wall infiltration by neoplastic clones and may be useful in the identification of high risk patients for cancer.

We conclude that there was a low prevalence of GBN in the population evaluated, with a higher occurrence among the elderly, and an association with gallbladder wall thickening. It is important to note that because this is a cross-sectional study, this paper is limited to suggesting associations, not being possible to determine causal relationships between the variables. In addition, the low incidence of this neoplasia makes it difficult to perform further statistical analyzes. Prospective and multicenter studies can remedy the limitations of this analysis.

R E S U M O

Objetivo: *descrever os achados histológicos das vesículas biliares de pacientes submetidos à colecistectomia e avaliar a presença de fatores associados ao câncer incidental da vesícula.* **Métodos:** *estudo descritivo, transversal e observacional de 1.278 exames anatomopatológicos de vesículas biliares oriundas de colecistectomias por colelitíase e de seus respectivos laudos, realizadas no período de janeiro de 2008 a dezembro de 2017.* **Resultados:** *o achado anatomopatológico mais frequente foi a colecistite crônica, presente em 1.251 pacientes (97,8%), seguido pela colesterolose em 131 (10,2%). O câncer de vesícula foi identificado em seis pacientes, com prevalência de 0,5% nesta amostra. Houve associação significativa entre a presença de câncer e idade ≥60 anos e com a espessura da parede ≥0,3cm.* **Conclusão:** *houve baixa prevalência de câncer de vesícula na população avaliada, maior ocorrência na população idosa e associação de tumor com espessamento da parede vesicular.*

Descritores: *Colelitíase. Colecistectomia. Neoplasias de vesícula biliar.*

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