DECREASED DENSITY OF INTERSTITIAL CAJAL-LIKE CELLS CORRELATE WITH CHOLELITHIASIS IN CHILDREN

ÇOCUKLARDAKİ KOLELİTİAZİSİN INTERSTİSYEL CAJAL-BENZERİ HÜCRE YOĞUNLUĞUNUN AZALMASI İLE İLİŞKİSİ

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Özet

Amaç: Çocuklardaki kolelitiazis örneklerindeki (interstisyel Cajal-benzeri hücre) ICLC yoğunluğun analiz edilmesi ve kontrol örneklerle karşılaştırılması amaçlanmıştır. Gereç ve Yöntem: Kolelitiazisli 20 ve kolesistitli 15 hastadan laparoskopik olarak safra keseleri rezekte edilmiştir. Doku örnekleri rutin histolojik inceleme için işlenmiş, ek olarak tüm kesitler immünohistokimyasal olarak CD117 ve Mast hücre triptaz antikorları ile boyanmıştır. Bulgular: Örnekler ICLC yoğunlukları açısından karşılaştırılmış ve kolelitiazisli grupta ortalama 23.30, kontrol grubunda ise 39.07 olarak saptanmıştır. ICLC ortalama sayılarındaki bu fark istatistiksel olarak anlamlı bulunmuştur (p=0.044). Tartışma: Safra taşı olan çocukların muskularis propriadaki (interstisyel Cajal hücresi) ICC veya ICLC yoğunlukları, safra taşı olmayan kontrol grubu çocukların örneklerine göre önemli ölçüde düşük bulunmuştur. Düz kas kontraksiyonu ile ilişkili olan ICC ve ICLC sayıları safra kesesi motilitesini etkileyebileceği düşünülmektedir. Bu çalışmada gözlenen histopatolojik farklılıkların çocuklardaki kolelitizasinin patofizyolojisinin açıklanmasında yardımcı olabileceği sonucuna varılmıştır.

Anahtar Kelimeler

Safra Taşı; İnterstisyel Cajal-Benzeri Hücre; Mast Hücresi; CD117/c-Kit; Safra Kesesi Motilitesi

Abstract

Aim: The present study aimed to analyze numbers of (Interstitial Cajal-like cells) ICLCs found in cholelithiasis specimens and compare them with controls specimens of children. Material and Method: Gallbladders were resected laparoscopically from 20 patients with cholelithiasis and 15 patients with cholecystitis. Tissue samples were processed for routine histological examination. Additionally, all sections were immunohistochemically stained with CD117 and Mast Cell Tryptase antibodies. Results: When specimens were compared for the density of ICLCs, mean number for the control group was 39.07, whereas for the cholelithiasis group it was found to be 23.30. The difference of means of ICLCs in these groups was found to be statistically significant (p=0.044). Discussion: We found that the density of (Interstitial cells of Cajal) ICC or ICLCs in the muscularis propria was significantly lower in specimens from children with gallstone disease than in specimens derived from the gallstone-free, which served as controls. Gallbladder motility may be affected by the number of ICC or ICLCs which are in interaction with smooth muscle contraction. The histopathological differences observed in this study may help to elucidate the pathophysiology of cholelithiasis in children.

Keywords

Gallstones; Interstitial Cajal Like Cells; Mast Cells; CD117/c-Kit; Gallbladder Motility

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Introduction

Cholelithiasis is the most common gallbladder disorder. Among the causes of cholelithiasis gallbladder hypomotility, mucus hypersecretion, and bacterial infections might be considered.

Interstitial cells of Cajal (ICC) are found along the entire gastrointestinal tract and contribute to regulation of gut motility [2,3]. Interstitial Cajal-like cells (ICLCs) are believed to be involved in innervation and motility, and a decrease in their number and/or density has been linked to a variety of intestinal motility disorders of the gallbladder [4,5,6]. We investigated the distribution of the ICLCs in the gallbladder because of their potential role in contributing to gastroenteric motility which on the other hand is also assumed to be essential in gallstone formation. Since ICLCs are involved in inducing smooth muscle contraction, a decrease in the density of these cells in the muscular layer of the gallbladder could induce gallbladder hypomotility and possibly lead to gallstone formation [4]. In recent years, several studies have been published regarding the ICLCs, performed experimentally or in specimens obtained from adults. However, to our knowledge, there has been no study published yet investigating the relationship between density of ICLCs and gallstone disease in children. The present study aimed to analyze numbers of ICLCs found in cholelithiasis specimens and compare them with cholecystitis-only (gallstone-free controls) specimens of children.

Material and Method

Gallbladders were resected laparoscopically from 20 patients with cholelithiasis and 15 patients with cholecystitis. The diagnosis of cholecystitis and symptomatic gallstone disease was made when cases presented clinically with crampy right upper quadrant abdominal pain radiating to the upper back or right shoulder (biliary colic) and confirmed by ultrasonographical investigation. Seven patients underwent cystectomy due to gallstones related to a hemolytic disease. Three patients were thalassemia major. Four patients were hereditary spherocytosis. All 35 patients underwent laparoscopic cholecystectomy with no conversion to open procedure. Cholecystitis-only specimens served as controls.

Tissue samples were processed for routine histologic examination with standard formalin fixation and paraffin embedding, and 5 µm thin sections were stained with hematoxylin-eosin. In addition, all sections (fundus, body, and neck of the three gallbladders) were immunohistochemically stained for CD117 (c-kit Oncoprotein) and Mast Cell Tryptase (AA1, 1:100, Thermo Scientific, USA) as follows: 3µm thin sections were cut, dried, and deparaffinized before placing them on the Ventana Benchmark GX immunostainer (Ventana, Tucson, AZ). Diaminobenzidine was used as a chromogen.

The antibody panel included CD117 (T595, ready-to-use, Leica, Newcastle, United Kingdom). We used skin sample as positive controls for CD117 and reactive lymph node for Mast Cell Tryptase. For negative controls, the primary antibodies were omitted. ICLCs were counted per 10 consecutive high-power fields (original magnification ×400; objective ×40, and eyepiece ×10) and means were calculated.

Mast cells which are mostly found in the mucosa and submucosa, and which also stained positive for CD117 were distinguished from ICLCs according to their morphological features (round or oval shaped and a centrally located nucleus) and by Mast Cell Tryptase staining, which is negative for ICLCs. Interstitial Cajal-like cells were predominantly spindle in shape (Fig).

Data are expressed as mean ± standard deviation. Chi-square test was used for statistical analysis, using SPSS v10.0 software (SPSS; Chicago, IL, USA). ***P*** values less than 0.05 were considered as statistically significant. Local ethics committee approval this study.

Results

The mean age for the control group (n=15) was 11, and 12.7 years for the study group (n=20). When specimens were compared for the density of ICLCs, mean number for the control group was 39.07±8.02, whereas for the cholelithiasis group it was found to be 23.30±3.50. The difference of means of ICLCs in these groups was found to be statistically significant (p=0.044). Cases with cholelithiasis had usually mean ICLCs numbers <30, whereas cases without a gallstone had >30. The distributions of ICLCs are given in detail in Table 1. Detail the distributions of CD117 positive ICLCs are shown figure.

Table 1. Distribution of ranked ICLCs numbers.

Number of ICLCs	Cholelithiasis (n)	Cholecystitis (n)	Total n of specimen
<20	3	-	3
21-30	16	1	17
31-40	1	9	10
41-50	-	3	3
>50	-	2	2
	20	15	35

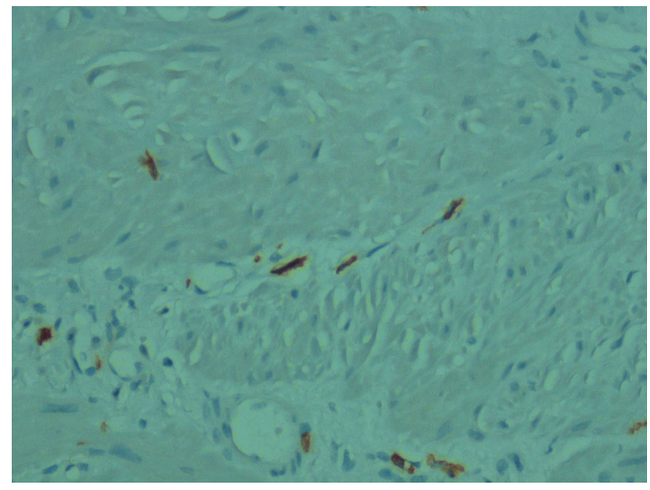

Figure. Distribution of CD117 positive ICLCs (CD117, X 400)

Discussion

Interstitial cells of Cajal (ICC) were first described by Ramón Santiago y Cajal in 1889 [3]. ICC or ICC-like cells that generate pacemaker activity are now being described in many muscular organs including the genitourinary tract, blood vessels, appendix, gallbladder and the uterus. Further studies successfully identified ICC or ICC-like cells by advanced techniques like electron microscopy and immunohistochemistry [7]. The ICC were detected predominantly within the muscularis propria, and they are regarded as important players for intestinal motility

which in case of their decrease also may point to their role in dysmotility conditions [2,8]. In pathologic conditions like motility disorders as observed in diabetic gastroenteropathy, slow-transit constipation, chronic idiopathic intestinal pseudo-obstruction, Hirschsprung's disease, Chagas disease, achalasia and hypertrophic pyloric stenosis the role of ICC has been recently investigated [8, 9, 10]. Ortiz-Hildago first suggested that ICLCs were present in the human gallbladder [3]. Previous studies and research were performed in guinea pig and murine models [5, 11]. Lavoie et al. have suggested the potential a role for ICLCs in the generation and propagation of spontaneous rhythmicity of the gallbladder [5].

Our study was performed in gallbladder specimens laparoscopically removed from children. In our study, we detected that the density of ICLCs in the muscularis propria was significantly lower in the patients with cholelithiasis than in cholecystitis (gallstone- free, controls). As to our knowledge, only one study has evaluated the ICLCs in the pathology of cholelithiasis but in adult specimens.

Arthur et al. examined the distribution of the ICLCs specimens from controls and patients with cholelithiasis with immunohistochemistry. They found a significant decrease in the density of ICLCs in cholelithiasis, even unrelated to the different stages of inflammation. They also evaluated cholesterol saturation index values in cholelithiasis and gallstone-free controls and found an increased cholesterol saturation index in the patients with gallstones, correlating with a lesser ICLCs density. Another important mechanism for the decrease of ICLCs in cholelithiasis was associated with chronic inflammation [8, 12].

Conclusion

In conclusion, we found that the density of ICC or ICLCs in the muscularis propria was significantly lower in specimens from children with gallstone disease than in specimens derived from the gallstone-free, which served as controls. Gallbladder motility may be affected by the number of ICC or ICLCs which are in interaction with smooth muscle contraction. Disrupting this interaction or a decrease in numbers of stimulating ICC or ICLCs would cause the smooth muscle surrounding the gallbladder function less effectively. The histopathological differences observed in this study may help to elucidate the pathophysiology of cholelithiasis in children.

Competing interests

The authors declare that they have no competing interests.

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Animal and human rights statements

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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