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

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Abstract
Aim: The present study aimed to analyze numbers of (Interstitial Cajal-like cells) ICLCs found in cholelithiasis specimens and compare them with controls of 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

Özet


Anahtar Kelimeler
Safra Taşı; İnterstisyel Cajal-Benzeri Hücre; Mast Hücreleri; CD117/c-Kit; Safra Kesesi Motilitesi

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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. Tryptase staining, which is negative for ICLCs. Interstitial Cajal-like cells were predominantly spindle in shape (Fig).

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>
<thead>
<tr>
<th>Number of ICLCs</th>
<th>Cholelithiasis (n)</th>
<th>Cholecystitis (n)</th>
<th>Total n of specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20</td>
<td>3</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>21-30</td>
<td>16</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>31-40</td>
<td>1</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>41-50</td>
<td>-</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>&gt;50</td>
<td>-</td>
<td>2</td>
<td>2</td>
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<td></td>
<td>20</td>
<td>15</td>
<td>35</td>
</tr>
</tbody>
</table>

Discussion
Interstitial cells of Cajal (ICC) were first described by Ramón Santiago y Cajal in 1889 [5]. 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 [2, 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.

**References**


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