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The enteric nervous system in patients with calculous and acalculous gallbladder

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ABSTRACT

Background: It is generally thought that gallbladder motility plays a more or less important role in the pathogenesis of gallstones. Some studies have shown that some abnormalities of its intrinsic innervations, but these studies were usually limited to one cell component.
Aims: We investigated the main cell components of gallbladder intrinsic innervation in patients with and without gallstones.
Methods: Archival gallbladder specimens from 39 patients, 27 with gallstones (age range 45–69 yrs) and 12 patients without gallstones (age range 39–71 yrs) were obtained. Full thickness sections were obtained from the gallbladder neck and immunohistochemistry was carried out for enteric neurons (neuron-specific enolase and calretinin), enteric glia (S100) and interstitial cells of Cajal (CD117 and CD34); tryptase staining was also done to distinguish the latter from mast cells.
Results: Apart from calretinin-positive neurons, patients with gallstones featured a significant decrease of neurons, enteric glial cells (EGC) and mast cells compared to those without gallstones; interstitial cells of Cajal were extremely few and only found in two patients, one for each group.
Conclusions: The intrinsic innervations of the gallbladder is abnormal in gallstone patients, and this may contribute to gallstone formation in these subjects.

1. Introduction

Gallstone formation is a frequent occurrence in Western adults, in whom there is an estimated prevalence of 10–15% [1]. The pathogenesis is due to a complex process, involving several (including genetic and environmental) factors [2]; among these, it is generally thought that gallbladder motility plays a more or less important role [1–3], and this motor function may be controlled/disrupted by a variety of neuro-humoral inputs [4]. Thus, as in other segments of the gastrointestinal tract, the intrinsic innervation (represented by the enteric nervous system, ENS) is likely to play a pivotal role in this context.

However, it is worth noting that studies assessing the various elements of the ENS in patients with gallstones are remarkably few in literature, and often only focusing on a specific aspect (e.g., the interstitial cells of Cajal, ICC) [5–8]. Therefore, there is paucity of information on the various components of the ENS in this setting.

Purpose of the present study was to evaluate some aspects of the ENS in the gallbladder of patients with and without gallstones.

2. Materials and methods

Archival samples of surgically excised gallbladders were obtained from 39 patients, 27 with cholesterol gallstones (7 men, 20 women, median age 53, range 45–69 yrs) and 12 patients without gallstones (5 men, 7 women, median age 5, range 39–71 yrs). Full-thickness samples were obtained from formalin fixed tissue and transversal sections obtained from the neck of the gallbladder after paraffin embedding and processed for both conventional histology (H&E) and immunohistochemistry (IHC). The neck area was chosen on the basis of previous studies showing that this part of the gallbladder features the higher number of nerve cells [9,10].

Concerning the various elements of the ENS, enteric neurons were assessed by both neuron-specific enolase (NSE, monoclonal

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antibody clone 22C9, Leica Microsystems Srl, Milano, Italy; dilution 1:200) and calretinin (polyclonal antibody, Histo-Line Laboratories, Pantigliate, Milano, Italy; dilution 1:250), the enteric glial cells (EGC) by S100 (polyclonal antibody, Leica Microsystems Srl, Milano, Italy; dilution 1:300), and the ICC by CD117 (polyclonal antibody, Dako, Carpinteria, CA; dilution 1:200). To distinguish ICC from mast cells, sections were also assessed by tryptase (monoclonal antibody clone AA1, Bio-Optica, Milano, Italy; dilution 1:4000). Moreover, to further characterize ICC and ICC-like cells, the so called telocytes [11], sections were also stained with CD34 (monoclonal antibody clone Qbend/10, Leica Microsystems Srl, Milano, Italy; dilution 1:200) which helps to identify this cell population [12].

For each patient, the number of immunopositive cells was calculated and expressed as the mean of cells on 10 well stained and well oriented microscopic fields for each region of interest at 40× magnification (Olympus BX 40, Tokyo, Japan). The slides were coded to ensure anonymity, and all calculations were made in blind by one of the authors unaware of the diagnosis.

Data between groups with and without gallstones were compared by means of the Mann–Whitney *U*-test, and are expressed in the text as medians (95%CI); values of $p < 0.05$ were chosen for rejection of the null hypothesis.

3. Ethical considerations

Dealing with a retrospective study, no individual patient identification was involved and no study-driven clinical intervention was performed; therefore, no ethical approval was necessary.

4. Results

The overall results are summarized in Table 1. The groups were balanced for gender and age. No patient died for gallstone disease. Concerning histopathological assessment, this revealed mild or no mucosal inflammation in patients without gallstones and moderate (7 patients, 35%) to severe (20 patients, 75%) inflammation in those with gallstones. Concerning the cell populations of the ENS, apart from calretinin-positive neurons, patients with gallstones featured a significant decrease of neurons, EGC and mast cells compared to those without gallstones (Fig. 1). Of interest, almost all CD117 positive cells were represented by mast cells, as confirmed by tryptase staining (Table 1, Fig. 2), and ICC were only present as 1–2 cells in a couple of patients (one for each group), as also confirmed by CD34 staining.

Table 1
Demographic and ENS variables in patients with and without gallstones.

	Gallstones	No gallstones	*P*
Sex	7 M, 20 F	5 M, 7 F	
Age	53 (45–69)	51 (39–71)	0.77
NSE	8 (7–8)	9 (8–10)	0.003
Calretinin	3 (3–3)	3.5 (2–5)	0.6
S100	10 (8–12)	15 (14–16)	0.001
CD117	14 (12–18)	25 (22–27)	0.0006
Tryptase	15 (13–19)	27 (23–29)	0.0005

5. Discussion

In recent years, there had been a rising interest in the investigation of the ENS in several different pathologic conditions of the gastrointestinal tract [13,14]. However, it is worth noting that there are relatively few such studies concerning the human gallbladder [15], and that these mostly focused on a single cell population [5–8,16]. Therefore, a more comprehensive investigation on the various components of the gallbladder ENS is needed and might add some useful data.

In the present study, we compared ENS findings obtained by immunohistochemical methods from surgical samples of patients with and without gallstones. We found that gallstone patients, compared with those without gallstones, had a significant decreases of enteric neurons, EGC and mast cells, suggesting that their gallbladders were functionally impaired. Therefore, these patients could have a motility defect as a pathophysiologic

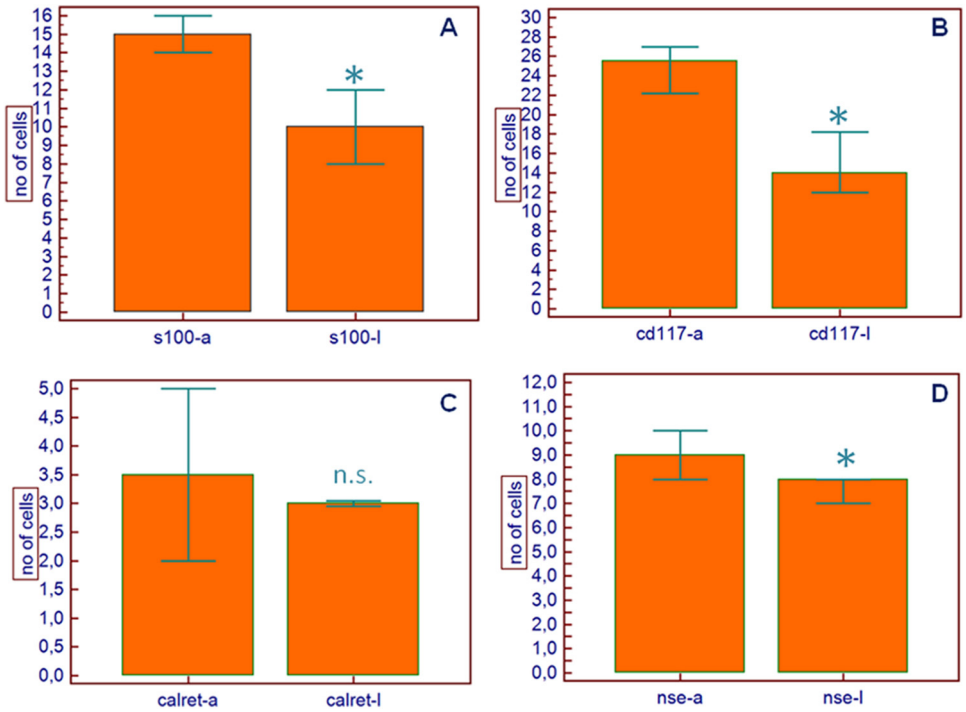

Fig. 1. The figure shows differences between enteric glial cells (S100), interstitial cells of Cajal (CD117) calretinin-positive neurons, and NSE-positive neurons in patients without (a) and with (l) gallstones.