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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

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GMN: Georgian Medical News is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board since 1994. GMN carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

GMN is indexed in MEDLINE, SCOPUS, PubMed and VINITI Russian Academy of Sciences. The full text content is available through EBSCO databases.

GMN: Медицинские новости Грузии - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения. Журнал индексируется в MEDLINE, отражён в базе данных SCOPUS, PubMed и ВИНИТИ РАН. Полнотекстовые статьи журнала доступны через БД EBSCO.

GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

ჟურნალი ინდექსირებულია MEDLINE-ის საერთაშორისო სისტემაში, ასახულია SCOPUS-ის, PubMed-ის და ВИНИТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებიდან.

WEBSITE

www.geomednews.com

К СВЕДЕНИЮ АВТОРОВ!

При направлении статьи в редакцию необходимо соблюдать следующие правила:

1. Статья должна быть представлена в двух экземплярах, на русском или английском языках, напечатанная через **полтора интервала на одной стороне стандартного листа с шириной левого поля в три сантиметра**. Используемый компьютерный шрифт для текста на русском и английском языках - **Times New Roman** (**Кириллица**), для текста на грузинском языке следует использовать **AcadNusx.** Размер шрифта - **12**. К рукописи, напечатанной на компьютере, должен быть приложен CD со статьей.

2. Размер статьи должен быть не менее десяти и не более двадцати страниц машинописи, включая указатель литературы и резюме на английском, русском и грузинском языках.

3. В статье должны быть освещены актуальность данного материала, методы и результаты исследования и их обсуждение.

При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

5. Таблицы необходимо представлять в печатной форме. Фотокопии не принимаются. **Все цифровые, итоговые и процентные данные в таблицах должны соответствовать таковым в тексте статьи**. Таблицы и графики должны быть озаглавлены.

6. Фотографии должны быть контрастными, фотокопии с рентгенограмм - в позитивном изображении. Рисунки, чертежи и диаграммы следует озаглавить, пронумеровать и вставить в соответствующее место текста **в tiff формате**.

В подписях к микрофотографиям следует указывать степень увеличения через окуляр или объектив и метод окраски или импрегнации срезов.

7. Фамилии отечественных авторов приводятся в оригинальной транскрипции.

8. При оформлении и направлении статей в журнал МНГ просим авторов соблюдать правила, изложенные в «Единых требованиях к рукописям, представляемым в биомедицинские журналы», принятых Международным комитетом редакторов медицинских журналов - http://www.spinesurgery.ru/files/publish.pdf и http://www.nlm.nih.gov/bsd/uniform_requirements.html
В конце каждой оригинальной статьи приводится библиографический список. В список литературы включаются все материалы, на которые имеются ссылки в тексте. Список составляется в алфавитном порядке и нумеруется. Литературный источник приводится на языке оригинала. В списке литературы сначала приводятся работы, написанные знаками грузинского алфавита, затем кириллицей и латиницей. Ссылки на цитируемые работы в тексте статьи даются в квадратных скобках в виде номера, соответствующего номеру данной работы в списке литературы. Большинство цитированных источников должны быть за последние 5-7 лет.

9. Для получения права на публикацию статья должна иметь от руководителя работы или учреждения визу и сопроводительное отношение, написанные или напечатанные на бланке и заверенные подписью и печатью.

10. В конце статьи должны быть подписи всех авторов, полностью приведены их фамилии, имена и отчества, указаны служебный и домашний номера телефонов и адреса или иные координаты. Количество авторов (соавторов) не должно превышать пяти человек.

11. Редакция оставляет за собой право сокращать и исправлять статьи. Корректура авторам не высылается, вся работа и сверка проводится по авторскому оригиналу.

12. Недопустимо направление в редакцию работ, представленных к печати в иных издательствах или опубликованных в других изданиях.

При нарушении указанных правил статьи не рассматриваются.

REQUIREMENTS

Please note, materials submitted to the Editorial Office Staff are supposed to meet the following requirements:

1. Articles must be provided with a double copy, in English or Russian languages and typed or compu-ter-printed on a single side of standard typing paper, with the left margin of **3** centimeters width, and **1.5** spacing between the lines, typeface - **Times New Roman** (**Cyrillic**), print size - **12** (referring to Georgian and Russian materials). With computer-printed texts please enclose a CD carrying the same file titled with Latin symbols.

2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

8. Please follow guidance offered to authors by The International Committee of Medical Journal Editors guidance in its Uniform Requirements for Manuscripts Submitted to Biomedical Journals publication available online at: http://www.nlm.nih.gov/bsd/uniform_requirements.html
http://www.icmje.org/urm_full.pdf
In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

9. To obtain the rights of publication articles must be accompanied by a visa from the project instructor or the establishment, where the work has been performed, and a reference letter, both written or typed on a special signed form, certified by a stamp or a seal.

10. Articles must be signed by all of the authors at the end, and they must be provided with a list of full names, office and home phone numbers and addresses or other non-office locations where the authors could be reached. The number of the authors (co-authors) must not exceed the limit of 5 people.

11. Editorial Staff reserves the rights to cut down in size and correct the articles. Proof-sheets are not sent out to the authors. The entire editorial and collation work is performed according to the author's original text.

12. Sending in the works that have already been assigned to the press by other Editorial Staffs or have been printed by other publishers is not permissible.

Articles that Fail to Meet the Aforementioned Requirements are not Assigned to be Reviewed.

ავტორთა საყურადღებოდ!

რედაქციაში სტატიის წარმოდგენისას საჭიროა დავიცვათ შემდეგი წესები:

1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე,დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx.** შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

3. სტატიაში საჭიროა გაშუქდეს: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

4. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: მიზანი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფჩხილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

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HELICOBACTER PYLORI AND GALLBLADDER PATHOLOGIES: IS THERE A CAUSE-AND-EFFECT RELATIONSHIP?

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Abstract.

Background and Objectives: The relationship between Helicobacter pylori infection and gallbladder diseases, particularly cholecystitis and gallbladder polyps, remains unclear. This study aimed to investigate the presence of H. pylori in gallbladder tissues and its potential role in gallbladder pathologies, as well as to examine the expression of chemokines CXCL2 and CXCL5 in these conditions.

Methods: A total of 137 laparoscopically excised gallbladders were analysed through histological examination, PCR for H. pylori-specific DNA, and quantitative real-time PCR for CXCL2 and CXCL5 gene expression. The study cohort included patients with acute calculous cholecystitis, chronic calculous cholecystitis, and gallbladder polyps.

Results: H. pylori was detected in 30.7% of cases by histological methods and 42.3% by PCR. Elevated expression of CXCL2 and CXCL5 was observed in 62% and 57.7% of cases, respectively, with a higher prevalence in acute cholecystitis compared to chronic conditions. However, no statistically significant association was found between H. pylori presence and the forms of cholecystitis, as well as between H. pylori presence and chemokine expression in gallbladder.

Conclusions: The study did not establish a direct link between the presence of H. pylori infection and forms of gallbladder pathologies. The findings suggest that other factors other than H. pylori may contribute to the upregulation of CXCL2 and CXCL5 in gallbladder diseases. Further research is needed to elucidate the complex interactions between H. pylori, chemokines, and gallbladder pathologies.

Key words. Helicobacter pylori, Gallbladder Disease, Cholecystitis, Chemokine CXCL2, Chemokine CXCL5.

Introduction.

Malignant tumors of the biliary tract, encompassing the gallbladder and bile ducts, present significant management challenges. Concurrently, both the incidence and mortality associated with these diseases are on an upward trajectory [1,2]. Early diagnosis is feasible in a mere 35% of cases, and in instances of delayed diagnosis, the median survival duration seldom surpasses twelve months [3]. Similar to gastric cancer, cancers of the hepato-biliary system predominantly arise against a backdrop of chronic inflammation, which may precipitate malignant transformations within the tissue [4].

Helicobacter pylori (H. pylori) is implicated as a causative agent of chronic and acute inflammation, as well as gastric cancer. Recent studies over the past decade have posited that the pathogenicity of Helicobacter pylori towards gastrointestinal organs is influenced by the bacterium's 2D protein profiles and the cytotoxin-associated gene (CagA). The latter encodes the CagA protein, thereby determining the variants of CagA isoforms [5,6]. It has been substantiated that the CagA protein, particularly its East Asian variant, escalates the risk of malignant progression [7].

Furthermore, Helicobacter pylori strains harboring "high-risk" CagA isoforms are considered primary instigators of cyclooxygenase-2 (COX-2) induction, a phenomenon observed in over 90% of gastric cancer instances [8]. Helicobacter pylori is deemed the principal cause of inflammatory processes involving COX-2 and prostaglandin E2 (PGE2), which are in turn linked to the expression of specific chemokines (e.g., CXCL5 and CXCL2) in gastric cells, as evidenced in gastric cancer scenarios [9].

However, a direct association between malignant inflammatory diseases of the biliary tract and Helicobacter pylori remains unestablished [2]. While correlations have been observed between gallbladder pathologies and the presence of H. pylori within gallbladder tissue [8,10,11], research validating a direct causality attributable to Helicobacter pylori is still lacking [12]. In vitro studies have demonstrated Helicobacter pylori's capacity to damage human gallbladder epithelial cells, potentially playing a significant role in calculous cholecystitis development [13]. Nonetheless, in vivo studies merely suggest an elevated risk of biliary tract diseases associated with H. pylori infection [2], typically confirmed via urease tests, ELISA for anti-H. pylori IgG and IgM [12], or PCR methods, particularly in countries with a high prevalence of hepatobiliary system pathologies, such as Japan, China, Pakistan, and Chile [11,12,14,15].

In Georgia, research into Helicobacter pylori spans over two decades, revealing a high prevalence among patients with gastritis (78%), peptic ulcer (58%), and gastric cancer (58%) [16], while also addressing critical diagnostic and treatment challenges [17-19]. Concerning Helicobacter pylori's link to biliary pathologies, hypotheses regarding two potential mechanisms of biliary tract infection have been proposed, alongside a conceptual framework for conducting related research [20]. Notably, no studies have yet been undertaken in Georgia to explore Helicobacter pylori's prevalence or role in the pathogenesis of gallbladder and bile duct cancer, as well as other inflammatory biliary diseases.

This work seeks to validate the hypothesis that Helicobacter pylori can induce specific inflammatory responses in the gallbladder and bile ducts, akin to its effects in the stomach. The study aims to ascertain the frequency of Helicobacter pylori infections in surgically removed gallbladders and to determine

the extent to which this bacterium contributes to gallbladder inflammation and its pathogenesis.

Materials and Methods.

Tissue Sampling: A total of 137 gallbladders, excised laparoscopically during 2021-2022, were examined. The patient age range was 22 to 90 years, with an average age of 55. The cohort consisted of 104 women and 33 men. Informed consent was obtained from all participants. The consent form, along with the research protocol, received approval from the Commission of Bioethics at the TSU Aleksandre Natishvili Institute of Morphology (Registration No. 3/20, Protocol No. 2, 2021).

Sample Collection and Preservation: Tissue samples were harvested from the excised gallbladders immediately after placement in a saline solution and then promptly transferred to containers filled with formalin. This approach effectively minimized ex-situ contamination of the samples with Helicobacter. We focused on collecting samples from both the neck and the fundus of the gallbladders, prioritizing the cervical regions due to the presence of mucous glands and a higher likelihood of encountering Helicobacter pylori. The samples were subsequently stored at -80°C in a specialized refrigerator for PCR analysis.

Histological and Molecular Analysis: Histological examination involved fixing gallbladder fragments in 10% buffered formalin (pH 7.2 – 7.3) and embedding them in paraffin. Sections (3-4 μm thick) from the paraffin blocks were stained using Giemsa's method, following the manufacturer's guidelines (Bio-Optica, Italy; MGG Stain, reference Code: 04-090805M). Helicobacter pylori was identified as grayish-brown rod-like microstructures on the mucous membrane epithelium and within gland lumens (Figure N1a,b). A ZEISS Primo Star light microscope (Germany) equipped with a digital camera (ZEN 2.3 SP1) was used for microscopic examination.

DNA Isolation and PCR Detection of H. pylori: DNA was isolated from formalin-fixed and paraffin-embedded (FFPE) gallbladder tissue using the QIAamp DNA FFPE Tissue Kit (Qiagen).

To determine the concentration of dsDNA in each DNA sample, we utilized the Qubit dsDNA BR Assay (Invitrogen™), and any measurement that was less than 2.0 ng was verified using the Qubit dsDNA HS Assay (Invitrogen™) [21].

H. pylori by RT-PCR: The presence of H. pylori was determined via real-time PCR using the ABI 7500 PCR system (Applied Biosystems). For detection of H. pylori in FFPE gallbladder tissue, we utilized primer and probe targeting 16S rRNA and Urea A genes. Both genes' primers and probes sequences were selected based on the study by Ramírez-Lázaro et al. [22]. Information about the corresponding primer-probe is provided in Table 1. A human beta-2 microglobulin (B2M) qPCR (Hs.PT.58v.18759587, IDT Inc.) served as an endogenous control. After the optimization, we applied 900 nM primer and 250 nM TaqMan probe concentrations. We run each sample in duplicate to ensure the reliability of the PCR reaction.

The amplification reaction was performed in TaqPath 1-Step Master Mix (No ROX) (Applied Biosystems™), and the thermal cycling condition was set according to the manufacturer's recommendations. Real-time PCR comprised a reverse transcription, 53°C for 10min, initial denaturation at 95°C for 2min, 40 cycles at 95°C for 15sec, and 60°C for 60sec. Each RT-PCR run incorporated a positive control (H. pylori-positive gastric biopsy), a negative control (endometrial tissue), and no-template controls to ensure the validity of the results.

CXCL2 and CXCL5 Gene Expression Study: We explored the gene expression of CXCL2 and CXCL5 chemokines in gallbladder tissues using the quantitative real-time RT-PCR method, akin to the approach employed for H. pylori-infected cells in the gastrointestinal tract [9]. The aim was to assess the expression levels of these chemokines, which are activated by COX-2 and PGE2 in H. pylori-infected gallbladder tissues.

RNA Isolation and Purity Assessment: Total RNA was isolated from formalin-fixed and paraffin-embedded (FFPE) gallbladder tissue samples utilizing the RNeasy FFPE Mini Kit (Qiagen), adhering to the manufacturer's validated protocol. For reference, we employed FFPE gallbladder samples devoid of

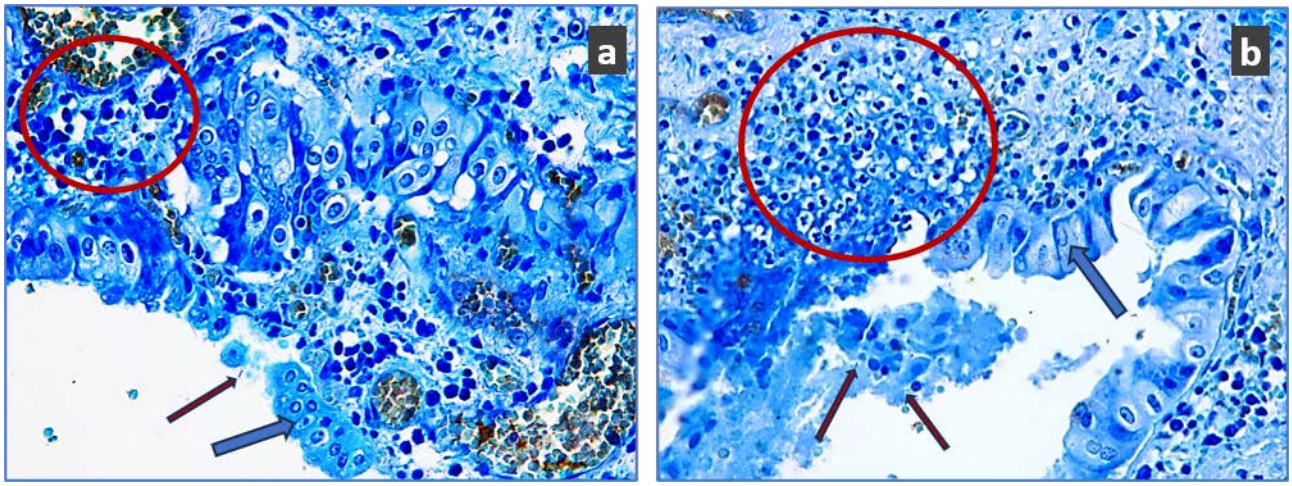

Figure 1. *a) Red circle - lymphocyte infiltrate; b) Red circle - neutrophil infiltrate; a,b) Blue arrow- epithelium of Gall Bladder; Red arrow- – Helicobacter Pylori.*

Table 1. *H.Pylori detection PCR primers.*

Gene	Forward primer	Reverse primer	Length (bp)
16S rRNA	5'-CTCATTGCGAAGGCGACCT-3'	5'-TCTAATCCTGTTTGCTCCCCA-3'	76
ureaA	5'-CGTGGCAAGCATGATCCAT-3'	5'-GGGTATGCACGGTTACGAGTTT-3'	77
TaqMan probe Sequence	**Sequence**		
16S RNA PROBE	5'-FAM-ATTACTGACGCTGATTGCGCGAAAGC-TAMARA-3'		
ureaA PROBE	5'-FAM-TCAGGAAACATCGCTTCAATACCCACTT-TAMARA-3'		

pathology (n=4), retrieved during Whipple procedures and right hemi-hepatectomies due to liver trauma.

To obtain high-yield mRNA from FFPE postoperative tissue, we used the RNeasy FFPE Mini kit (Qiagen), which includes deparaffinization, protease K digestion, and DNase treatment steps in the protocol. Quantitative and qualitative assessment of isolated RNA was performed by Qubit technology, more precisely via RNA XR Qubit assay and Qubit QC Assay Kit to determine the degree of RNA degradation and to measure RNA concentration respectively.

Quantitative Real-Time PCR Analysis: The qRT-PCR analysis of CXCL5 and CXCL2 gene expression was performed using the ABI 7500 PCR system from Applied Biosystems. We employed TaqMan Gene Expression assays for CXCL5 (ID-Hs01099660_g1) [23] and CXCL2 (ID-Hs00601975_m1) [24] from Applied Biosystems, with human 18S ribosomal RNA (ID- Hs03928990_g1) [25] as the normalization control. Individual patient samples for each assay were run in duplicate.

We set up a single plex PCR experiment mixture for corresponding targets (CXCL2, CXCL5, and 18S). Thus, Rnx for a single target has been prepared as follows: 6.25µL TaqPath 1-Step Multiplex Master Mix (No ROX) (Applied Biosystems™), 1µL real-time PCR assay, 7.75µL nuclease-free water, and 10µL RNA sample. The thermal cycling conditions recommended by the manufacturer were adhered to, encompassing UNG incubation, at 25°C for 2sec, reverse transcription at 53°C for 10sec, 40 amplification cycles of polymerase activation at 95°C for 2sec, and elongation at 60°C for 60sec. No template controls were included in each run.

Data Analysis: Relative gene expression was ascertained using CT values, with the $2^{-\Delta\Delta CT}$ method applied to calculate relative changes in gene expression. Statistical analyses of the findings were conducted using SPSS software (version 25.0, SPSS Inc., Chicago, IL). Chi-square test was employed to evaluate the potential association between Helicobacter pylori infection and gallbladder disease. ANOVA test was used to evaluate the relationship between gallbladder disease and the molecular-level inflammatory responses to cytokines and chemokines. The Student t-test was used to assess the association between the presence of Helicobacter pylori DNA and CXCL2 and CXCL5 expression levels. A 95% confidence interval was utilized to determine the reliability of the results.

Results.

Histopathological Findings: The histopathological analysis of 137 laparoscopically excised gallbladders revealed acute cholecystitis in 79 cases, with acute calculous cholecystitis identified in 77 patients and acute non-calculous cholecystitis - in 2 patients. Chronic calculous cholecystitis was diagnosed in 53 patients, and gallbladder polyps were confirmed in 5 cases.

Helicobacter Detection: Helicobacter presence was histologically confirmed in 42 cases (30.7%) using the Giemsa staining method. Among patients with acute calculous cholecystitis, Helicobacter was detected in 22 cases (28.6%), and in 20 patients (37.7%) with chronic calculous cholecystitis. No Helicobacter pylori presence was observed in cases of acute non-calculous cholecystitis or gallbladder polyps.

PCR Analysis: The specific DNA of Helicobacter pylori was identified in 58 cases (42.3%) through PCR testing. Of these, 33 patients (42.9%) had acute calculous cholecystitis, and 23 patients (43.4%) had chronic calculous cholecystitis. Helicobacter Pylori-specific DNA was also found in single cases of acute non-calculous cholecystitis and gallbladder polyps, representing 50% and 20% of these cases, respectively (refer to Table N2).

Table 2. *Distribution of Helicobacter Pylori DNA test results according to gallbladder histopathological diagnoses.*

Histopathological diagnosis	**Confirmation of the presence of Helicobacter Pylori-specific DNA by PCR**		
	Positive	**Negative**	**Total**
Acute calculous cholecystitis	33	44	**77**
Acute noncalculous cholecystitis	1	1	**2**
Gallbladder polyps	1	4	**5**
Chronic calculous cholecystitis	23	30	**53**
Total	**58**	**79**	**137**

Chemokine Gene Expression: In the gallbladder tissues, CXCL2 was found to be elevated in 85 cases (62%), and CXCL5 expression was increased in 79 cases (57.7%). Both genes showed elevated expression in 67 cases (48.9%). Specifically, in acute calculous cholecystitis, CXCL2 expression was noted in 56 cases (72.7%), and CXCL5 in 58 cases (75.3%). Both chemokines showed 100% elevated expression in the two cases of acute non-calculous cholecystitis. In chronic calculous cholecystitis, CXCL2 and CXCL5 expressions were observed in 23 cases (43.4%) and 18 cases (34%), respectively. Among gallbladder polyp cases, CXCL2 expression was found in 4 cases (80%), and CXCL5 in 1 case (20%) (see Table N3).

Relationship with Helicobacter Pylori: Despite the PCR confirmation of Helicobacter Pylori-specific DNA in 58 patients, 45 out of 85 cases with increased CXCL2 expression (52.9%) and 40 out of 79 cases with elevated CXCL5 expression (50.6%) (see Table N4).

Table 3. *Distribution of chemokine gene expression cases according to histopathological diagnoses of the gallbladder.*

Histopathological diagnosis	**Increased expression of chemokine genes**		
	Increased expression of CXCL2 gene	**Increased expression of CXCL5 gene**	**Total**
Acute calculous cholecystitis	56	58	**77**
Acute noncalculous cholecystitis	2	0	**2**
Gallbladder polyps	4	1	**5**
Chronic calculous cholecystitis	23	18	**53**
Total	**85**	**79**	**137**

Table 4. *Distribution of Helicobacter Pylori-specific DNA Study Results in Relation to CXCL2 and CXCL5 Chemokine Gene Expression.*

Increased expression of chemokine genes	**Confirmation of the presence of Helicobacter Pylori-specific DNA by PCR**	
	Positive	**Negative**
Increased expression of CXCL2 genes - 85 cases	40	45
Increased expression of CXCL5 genes - 79 cases	39	40
Increased expression of both genes together - 67 cases	33	34

Statistical Analysis: The $\chi 2$ analysis did not establish a statistically significant association between the forms of gallbladder diseases and Helicobacter infection based on both histological and PCR data ($p=0.3$ and $p=0.8$, respectively). However, the ANOVA test revealed significant relation between gallbladder diseases and the expression of CXCL2 ($p=0.03$) and CXCL5 ($p<0.001$), indicating that gene expression of both chemokines is notably elevated in cases of acute cholecystitis compared to chronic cholecystitis. However, the Student's t-test revealed that there is no statistically significant association between Helicobacter pylori DNA presence and CXCL2 expression levels ($p = 0.102$). For CXCL5 expression levels, the Student's t-test indicated a marginal association with Helicobacter pylori DNA presence ($p = 0.06$).

Discussion.

In this study, we investigated the prevalence of Helicobacter pylori and the expression of CXCL2 and CXCL5 chemokine genes across various gallbladder pathologies. Although no direct association was established between gallbladder disease and Helicobacter infection, a significant association was identified between gallbladder disease and chemokine gene expression.

Among the 85 gallbladder tissue samples exhibiting elevated CXCL2 gene expression, Helicobacter pylori was detected in only 40 cases through PCR. Similarly, of the 79 samples with increased CXCL5 gene expression, Helicobacter pylori presence was confirmed in 39 cases by PCR. Furthermore, in 67 samples showing elevated expression of both genes, Helicobacter pylori was identified in just 33 cases via PCR.

The absence of Helicobacter pylori in cases with heightened chemokine gene expression may be attributed to various biological and pathological factors. For instance, the upregulation of chemokines could result from other bacterial or viral infections that incite inflammatory responses within the gallbladder or adjacent tissues. While bacterial infections are commonly linked to gallbladder inflammation, the role of viral infections cannot be discounted [26]. Moreover, increased chemokine expression might stem from non-infectious inflammation [27] triggered by gallstones, biliary sludge, or autoimmune reactions, wherein the immune system's activation leads to elevated chemokine levels. Additionally, systemic inflammatory responses or coexisting diseases might contribute to this phenomenon [28].

Chemokines such as CXCL2 (also known as MIP2-alpha) and CXCL5 (ENA-78) play crucial roles in recruiting and activating neutrophils, pivotal in inflammation [29]. The heightened expression of these chemokines in gallbladder tissues suggests their potential involvement in cholecystitis pathogenesis, hinting at new therapeutic or preventive strategies targeting these chemokines.

While the increased expression of CXCL2 and CXCL5 in gallbladder tissues does not directly relate with Helicobacter pylori prevalence, their coexistence in approximately 47.1-49.4% of cases does not entirely negate a possible cause-and-effect relationship. Future research on a larger scale is warranted to further explore the potential link between elevated CXCL2 and CXCL5 expression in gallbladder tissues and the presence of Helicobacter pylori. The same might be assumed regarding the link among the Helicobacter pylori and cholelithiasis in both acute and chronic cholecystitis, taking into the account, that H. pylori were detected in approximately 43% of the patients with the gallstones. This recommendation aligns with findings from a systemic review and meta-analysis examining the association between Helicobacter pylori infection in the gallbladder and conditions like chronic cholecystitis and cholelithiasis [2].

Potential Mechanisms by Which H. pylori Affects Biliary Diseases:

The interplay between Helicobacter pylori infection and biliary diseases is complex, encompassing various potential mechanisms. H. pylori-induced inflammation within the stomach and duodenum might indirectly influence the biliary system, contributing to conditions such as chronic cholecystitis. The infection may alter bile composition, precipitate gallstone formation, or impair bile function [14,30]. Moreover, the immune response elicited by H. pylori could extend its effects to the biliary system, although the precise mechanisms warrant further exploration [31].

Detection of H. pylori in Gallbladder Tissue:

The detection of Helicobacter pylori in gallbladder tissue presents a notable challenge. Helicobacter pylori thrives in the stomach's acidic environment by neutralizing gastric acid, which facilitates its survival within the gastric mucosa. The presence of H. pylori in regions outside the stomach, such as the bile or gallbladder, introduces intriguing questions regarding its survival mechanisms in varied pH environments. Nevertheless, the detection of H. pylori in the gallbladder mucosa is strongly associated with its presence in the stomach, suggesting a complex relationship between gastric and biliary H. pylori infections [32].

Challenges in Confirming H. pylori Colonization:

It remains challenging to ascertain whether the detection of Helicobacter DNA in the liver signifies actual microbial colonization or merely indicates the entero-hepatic circulation of Helicobacter and/or its DNA [31]. The difficulty in culturing Helicobacter from bile ducts suggests that, in some cases, bile from the portal circulation may contain Helicobacter DNA but not viable bacteria, detectable through PCR. This hypothesis is supported by our data, which showed Helicobacter pylori detection rates of 30.7% by Giemsa staining and 42.3% by PCR, The higher frequency of detection of Helicobacter Pylori by the PCR method compared to the detection by the Giemsa method is noted by other authors as well [15]. However, there are studies, according to which the frequency of detection of Helicobacter Pylori by histological and molecular-biological examinations is approximately equal [33] The above highlights a discrepancy that necessitates further refinement and standardization of detection methods.

Routes of H. pylori Entry into the Biliary System:

Helicobacter pylori may access the biliary system through two primary routes: the ascending route via the papilla of Vater and the enterohepatic translocation route via portal blood or lymph [20,31,34]. The papilla of Vater, or the major duodenal papilla, and the sphincter part of the bile duct, distinguished by their abundant mucosal folds and muscle fibers, form independent sphincters for the bile and pancreatic ducts. These sphincters regulate bile and pancreatic juice flow into the duodenum, bile accumulation in the gallbladder, and prevent reflux from the duodenum into the pancreas and vice versa. The complex sphincter system at the terminal part of the common bile duct, particularly the sphincter of Oddi, makes it difficult to envision H. pylori's ascending penetration into the bile ducts without sphincter dysfunction. Moreover, such dysfunction could allow not only H. pylori but also other gastrointestinal microbes to enter the liver and bile ducts, further complicating the investigation of H. pylori's role in hepatobiliary pathologies.

Bacterial Translocation and Its Implications:

Bacterial translocation involves several pathogenic factors, including intestinal microflora overgrowth, intestinal mucosal damage, immune system impairment, and endotoxemia. Bile's inhibitory effect on intestinal bacteria growth underscores the antimicrobial properties of bile acids and their salts [35,36], particularly against anaerobic gram-negative microorganisms [37]. Bacterial translocation is observed in conditions such as mechanical jaundice and various intestinal pathologies, leading to increased systemic circulation of Gram-negative bacteria like E. coli and their endotoxins.

Recent scientific literature suggests that bacteria and endotoxins from damaged intestinal epithelia can bypass the liver and enter the systemic circulation, as evidenced in conditions like extrahepatic cholestasis [36]. This phenomenon raises the possibility that H. pylori, similar to E. coli, might undergo translocation and reach the liver and bile ducts through portal or lymphatic routes. However, such translocation likely results from pre-existing biliary pathologies rather than causing them, which limits the usefulness of these observations in elucidating the role of translocated microbes in hepatobiliary disease development.

The participation of translocated microbes, including various Helicobacter species, in the initiation and pathogenesis of hepatobiliary system pathologies remains an open question, necessitating further clinical and experimental studies. Understanding the complex mechanisms by which H. pylori interacts with the biliary system and the challenges associated with its detection and colonization confirmation will be crucial in advancing our knowledge of hepatobiliary diseases and developing targeted therapeutic strategies.

The limitations of the study are primarily attributed to the relatively low number of cases examined. Nevertheless, it serves as one of the initial steps in testing the hypothesis regarding the involvement of H. pylori in the pathogenesis of gallbladder diseases.

Conclusion.

Our research findings do not establish a direct causal relationship between Helicobacter pylori infection and gallbladder pathologies. The absence of a statistically significant association between the forms of gallbladder diseases and Helicobacter pylori, as determined by both histological and PCR analyses, suggests that changes observed in the gallbladder may not be directly attributable to H. pylori infection. While a direct association was observed between gallbladder disease and elevated expression of the chemokines CXCL2 and CXCL5 in cases of acute cholecystitis compared to chronic cholecystitis, no direct relation between the expression of these chemokines and H. pylori-specific DNA was established. This finding implies the involvement of additional factors in the upregulation of these chemokines.

Furthermore, the concurrent presence of CXCL2 and CXCL5 with H. pylori in 47.1-49.4% of the cases does not definitively rule out a potential cause-and-effect relationship between them. Therefore, the role of H. pylori in the etiology of gallbladder diseases remains an open question, warranting further investigation to clarify its involvement conclusively.

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Conflict of Interest:

Authors declare no Conflict of Interest for this article.

Author Contributions:

Study concept and design (LG, VG, DK), acquisition of data (LG, ZB, TT), analysis and interpretation of data (LG, KT, ZB, IJ, NG, NC, TS, DK), drafting of the manuscript (DK, TT, ZB, LS), statistical analysis (LS), critical revision of the manuscript for important intellectual content (LG, VG, DK), administrative and technical support (NG, DK). All authors have made a significant contribution to this study and have approved the final manuscript.

Ethical Statement:

This study was carried out in accordance with the recommendations of Helsinki Declaration. Informed consent was obtained from all participants. The consent form, along with the research protocol, received approval from the Commission of Bioethics at the TSU Aleksandre Natishvili Institute of Morphology (Registration No. 3/20, Protocol No. 2, 2021).

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