Research Article

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Proof of the association between adenovirus infection and appendicitis in children through pathological evidence.

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ABSTRACT

Background : Appendicitis is inflammation and swelling of the appendix that can occur at any age. It can be caused by fecalith blocking the appendix entrance and has been associated with lamina propria thickening due to infection. Some reports have observed a relation between appendicitis and hyperplastic lymphoid tissue, which might be caused by infections like adenovirus, while others have linked adenovirus infection and appendicitis in children.
Methods : We performed a retrospective pathology review of tissue samples from 120 children aged <18 years who underwent an appendectomy in patients with appendicitis at Cathay General Hospital between January 2016 and January 2021. Pathological tissues from appendectomies were processed into formalin-fixed paraffin embedding (FFPE). Adenovirus immunohistochemistry (IHC) and quantitative polymerase chain reaction (qPCR) were analyzed to determine the positive rate of adenovirus in appendicitis.
Results : Extracted DNA quality was assessed at an optical density (OD) ratio of 260 nm and OD 280 nm (≥1.8). Concentrations ranged from 91–1087 ng/μL. None of control subjects were positive for IHC with anti-adenovirus antibody and qPCR analysis (Cq ≤40). However, the positive rates were significantly higher in patients by IHC detection (25.8%, 31 of 120; P = 0.010) and qPCR analysis (35.0%, 42 of 120; P = 0.002)
Conclusion : Our study directly confirmed the relationship between adenovirus infection and appendicitis using retrospective pathology evidence. IHC analysis and qPCR detection confirmed evidence of adenovirus infection in patients with appendicitis. Further, qPCR is as useful and reliable as IHC for diagnosing adenovirus in appendicitis, showing higher sensitivity compared with IHC.

Keywords
Aenovirus ; Apendicitis ;Imunohistochemical analysis ; Qantitative polymerase chain reaction

LIST OF ABBREVIATIONS

IHC(immunohistochemistry), qPCR (quantitative polymerase chain reaction), FFPE (formalin fixed paraffin embedding)

INTRODUCTION

Appendicitis is inflammation and swelling of the appendix that can occur at any age. It can be caused by fecalith blocking the appendix entrance and has been associated with lamina propria thickening due to infection. Some reports have observed a relation between appendicitis and hyperplastic lymphoid tissue, which might be caused by infections like adenovirus, while others have linked adenovirus infection and appendicitis in children. This study retrospectively analyzed appendix tissue pathology from children who underwent appendectomy and more directly investigate the relation between adenovirus infection and appendicitis. We included 120 children under age 18 who underwent appendectomy in patients with appendicitis at Cathay General Hospital between January 2016 and January 2021. Adenovirus

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immunohistochemistry (IHC) and quantitative polymerase chain reaction (qPCR) were analyzed to determine the positive rate of adenovirus in appendicitis. Thirty-one cases (25.8%) were positive for anti-adenovirus antibody IHC. qPCR was positive for adenovirus in 42 cases (35%). Eleven (9.2%) tested positive by both IHC and qPCR. Our pathological evidence directly confirms a relation between adenovirus infection and appendicitis. IHC and qPCR analyses confirmed evidence of adenovirus infection in patients with appendicitis. qPCR is as useful and reliable as IHC, and more sensitive, for diagnosing adenovirus in appendicitis.

While adenoviruses are a rarely reported cause of acute appendicitis in children, they are a well-recognized cause of intussusception in infants and young children (1-4). Human adenoviruses are classified into seven species (A–G) and 52 recognized serotypes. Species B (primarily serotypes 3, 7, 11, 14, and 21) and species E (serotype 4) are most often associated with epidemic acute respiratory illness. Appendicitis is also associated with a viral prodrome that precedes its initial symptoms. The presumed pathophysiology is virally induced lymphoid hyperplasia leading to obstruction of the appendix lumen. Lymphoid hyperplasia is defined as crowding of follicles above average size, with prominent reactive germinal centers associated with squamous epithelium, which increases the number of intraepithelial lymphocytes and narrows the lumen. In addition to the fecaliths etiology, appendicitis can occur secondary to appendiceal lymphoid hyperplasia leading to ischemic necrosis, especially at its tip, and subsequent suppurative appendicitis. The goal of the retrospective pathology analysis herein was to directly test evidence of a relation between adenovirus infection and appendicitis in children.

METHODS

Study Participants

We performed a retrospective pathology review of tissue samples from 120 children aged <18 years who underwent an appendectomy in patients with appendicitis at Cathay General Hospital between January 2016 and January 2021. Pathological tissues from appendectomies were processed into formalin-fixed paraffin embedding (FFPE). Adenovirus immunohistochemistry (IHC) and quantitative polymerase chain reaction (qPCR) were analyzed to determine the positive rate of adenovirus in appendicitis. There were 20 cases in the control group, including half males and half females. The time is also from January 2016 to January 2021. These were patients under the age of 18, and appendiceal tissue from non-appendicitis patients was studied and analyzed.

Immunohistochemical Analysis

Serial 4 μm paraffin sections were deparaffinized using EZ prep (Ventana Medical Systems, Inc., Tucson, AZ, USA). Slides were incubated with anti-adenovirus (Bio SB, Santa Barbara, CA, USA) in a 1:50 titration for 32 min using the automated Ventana BenchMark XT (Ventana Medical Systems, Inc.). Labeling was detected with the OptiView DAB Detection Kit (Ventana Medical Systems, Inc.) according to the manufacturer's protocol. All sections were counterstained with hematoxylin in Ventana reagent. Immunohistochemically stained slides were reviewed, and their correlation with histologic interpretations of a pathology specialist were analyzed.

Quantitative PCR

DNA was extracted from 4 μm sections of FFPE tissue samples using the GeneRead DNA FFPE kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. HAdV qPCR assay was used to detect adenovirus species A–F. The primers and probes used for HAdV qPCR are listed in the legend of Figure 2. A 10 μL reaction mixture included 5 μL of 2X QuantiNova Probe PCR Master Mix (QIAGEN, Germany), 0.4 μM of each primer, 0.2 μM probe, and 1 μL FFPE DNA. The qPCR assay was performed with the LightCycler 96 Instrument (Roche Diagnostics, Rotkreuz, Switzerland). The PCR protocol was 1 cycle at 95 ^{O}C for 2 min, followed by 60 cycles at 95 ^{O}C for 5 sec each and then at 60 ^{O}C for 30 sec. For human adenovirus hexon-specific primers and probes, refer to the report by Wong et al (5). A quantification cycle (Cq) value >40 was considered negative.

Statistical analysis

The positive rates of adenovirus IHC and qPCR from 140 subjects (120 patients with appendicitis and 20 control individuals without appendicitis) were compared using the chi-squared test. All statistical analyses were performed with IBM SPSS Statistics for Windows (Version 22.0; IBM Corp.). $P < 0.05$ was considered to indicate a statistically significant difference.

RESULTS AND DISCUSSION

Extracted DNA quality was assessed at an optical density (OD) ratio of 260 nm and OD 280 nm (≥1.8). Concentrations ranged from 91–1087 ng/μL. None of control subjects were positive for IHC with anti-adenovirus antibody and qPCR analysis (Cq ≤40) (Table 1). However, the positive rates were significantly higher in patients by IHC detection (25.8%, 31 of 120; $P = 0.010$) (Table 1 & Figure 1)and qPCR analysis (35.0%, 42 of 120; $P = 0.002$) (Table 1 & Figure 2).

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TABLE 1 : Positive rates of immunohistochemical staining and quantitative PCR for adenovirus from appendix of children with appendicitis.

Tests	Control	Patient	P value
Numbers	20	120	
IHC			
Positive (%)	0 (0.0)	31 (25.8)	0.010
Negative (%)	20 (0.0)	89 (74.2)	
qPCR			
Positive (%)	0 (0.0)	42 (35.0)	0.002
Negative (%)	20 (0.0)	78 (65.0)	

Note: Age of subjects < 18 years old; IHC, immunohistochemistry; qPCR, quantitative PCR.

Figure 1 :

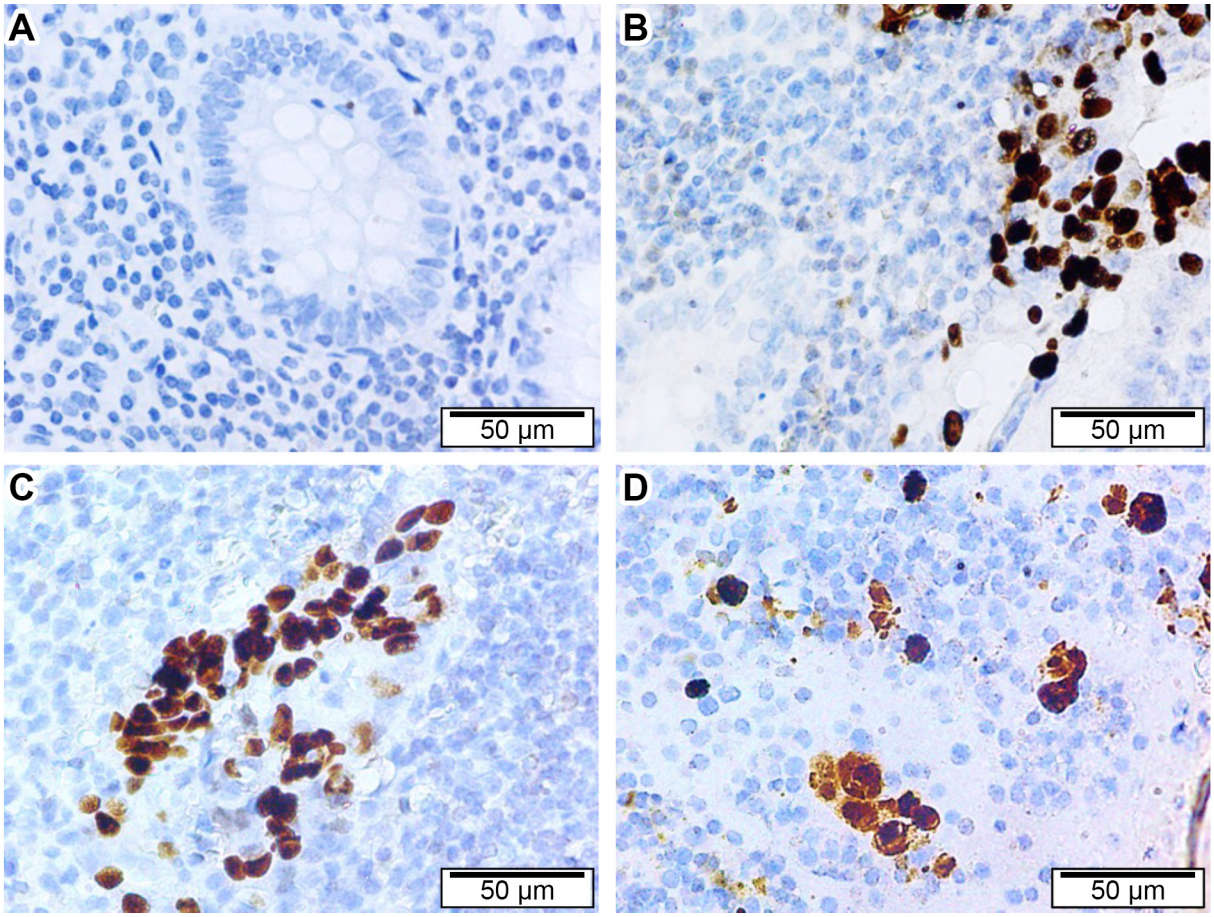

FIGURE 1 Representative images of immunohistochemical staining for adenovirus from appendix of children with appendicitis. (A) case with negative IHC signal, (B-D) cases with different positive types of IHC signal, which showing abundant adenovirus antigens in appendix epithelial cells. IHC, immunohistochemical staining. Scale bar, 50 μm.

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Figure 2:

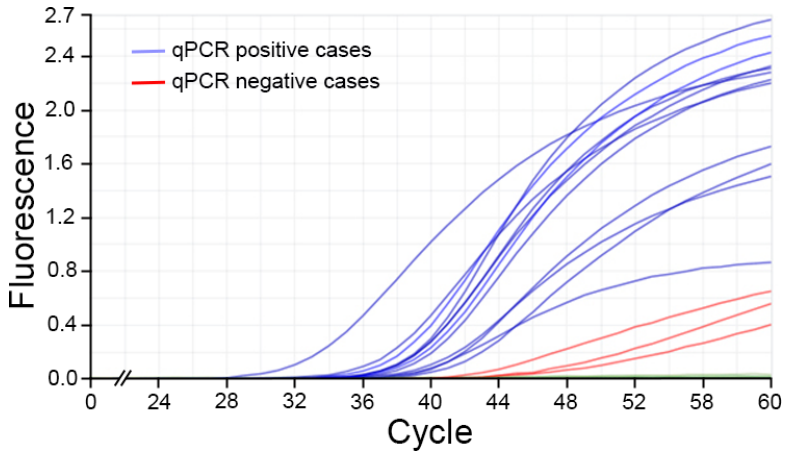

FIGURE 2 Quantitative PCR for adenovirus from appendix of children with appendicitis. qPCR revealed that approximately 35.0% (42 of 120) of DNA samples were positive (Cq≤40). The 10 upper-right S-shaped curves (blue) and 3 lower-right slope lines (red) represent qPCR-positive and qPCR-negative results, respectively. Primer sequences for qPCR: forward, 5′-CAGTGGKCDTACATGCACATC-3′; reverse, 5′-GCGGGCRAAYTGCACSAG-3′. Probe sequence, 5′-FAM-CTCAGGTACTCCGARGC-MGB-NFQ-3′. K=G, T; D=A, G, T; R=A, G; Y=C, T; S=C, G. FAM, TaqMan probe; MGB, minor groove binder; NFQ, non-fluorescent quencher.

We retrospectively analyzed pathological evidence from 120 children aged <18 years who underwent appendectomy, which confirmed the relationship between adenovirus infection and appendicitis through IHC and qPCR analyses.

Adenoviruses are important human pathogens associated with a wide range of clinical diseases. Among these, the association between adenovirus infections and intussusception has been well documented (6). For example, a team from our hospital showed this relation by analyzing the annual incidences of both conditions from January 2008 to June 2011, showing a similarity in peak months for both (4). Another study showed simultaneous intussusception and adenovirus infection in monozygotic twins (2).

In addition to intussusception, adenoviruses can induce appendicitis. Adenoviruses of different serotypes can cause both intussusception (species C serotypes 1, 2, 5, and 6) and acute appendicitis (species B, mainly serotypes 3, 7, 11, 14, 21, and species E serotype 4) (1, 7). The cause of appendicitis is usually obstruction of the appendiceal lumen, resulting in progressively increased intraluminal pressure and subsequent venous congestion that leads to progressive inflammatory changes and ischemia. Most hypotheses about the mechanism of appendicitis rely on the concept of appendix blockage. Although this seems reasonable, experimental evidence suggests that the role of feces as an etiology of appendicitis is limited (8). Obstruction occurs in 50–80% of appendicitis cases and, in children, is most often due to fecaliths or lymphoid hyperplasia. However, the most common cause of lymphoid hyperplasia is catarrhal inflammation secondary to viral infection.

The incidence of acute appendicitis is estimated at 75 per 100,000 per year, with the highest rate among children aged 10–19 years. Overall, 67% of cases are nonperforated (9). Historically, the cause of appendicitis has been believed to be feces blocking the lumen. However, our experience has been that a bezoar is not always present, which we were able to show in approximately 50% of patients. Lynch et al. also noted that adenovirus-associated acute appendicitis is underrecognized (7).

Regarding the relationship between lymphoid hyperplasia and appendicitis, the general theory is that lymphoid hyperplasia may lead to appendix obstruction, followed by suppurative appendicitis. The concept of lymphoid hyperplasia associated with appendicitis is not new and numerous reports have dealt directly or indirectly with the topic (10-13). A recent article by Sheridan et al. focused on this issue (14) and confirmed our study of appendicitis without fecalith (15, 16). Further, the United States (US) Food and Drug Administration approved an adenovirus vaccine in 2011 (only available to US military personnel aged 17–50 years), the indication of which is prevention of febrile acute respiratory diseases caused by adenovirus types 4 and 7, and indirectly to reduce the occurrence of appendicitis (7).

Spectrophotometry can be used to measure microgram quantities of pure DNA samples (i.e., DNA uncontaminated by proteins, phenol, agarose, or RNA). Fluorescence is more sensitive and can measure DNA in nanogram quantities. Absorbance readings can be used to determine sample concentration and calculate the A260/A280 ratio to indicate sample purity. It is generally accepted that relatively pure DNA will yield an A260/A280 ratio ≥1.8 (17). All samples herein met this standard.

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IHC plays an important role in diagnosis of infectious diseases in tissue samples. IHC using monoclonal and polyclonal antibodies is useful for detecting pathogen antigens in fixed tissues, complementing the direct diagnosis of infectious diseases by fresh tissue PCR and culture (18). While conventional PCR assays have been described for adenoviruses based on hexon and fiber gene sequences, these are labor intensive due to the need for post-PCR product analysis by gel electrophoresis and confirmatory hybridization assays or sequencing (19). Our findings also support qPCR assay for sensitive and specific detection of adenoviruses.

To our knowledge, this is the first report of adenovirus infection clinically manifesting as acute appendicitis without intussusception in Taiwan. Here, we combined the results of IHC and qPCR to show that the adenovirus detected in this study was species B serotype 3 (5). Our study confirmed that adenovirus can infect the appendix, presenting as acute symptoms and signs of appendicitis. We hypothesize that adenoviruses may play a causative role in some acute appendicitis cases. Future experiments will be needed to examine appendicitis cases for the presence of infectious agents as potential mechanisms. One limitation of this study is a second pathologist's interpretation of samples was not included. Finally, interpreting Cq ≤40 as positive may require some adjustment.

CONCLUSION

Appendicitis appears to occur when the appendix becomes blocked by hard fecal material (bezoars) or swollen lymph nodes in the intestine, which can occur with various infections.Here, we explore the relationship between adenovirus infection and appendicitis using retrospective pathological evidence.IHC analysis and qPCR testing confirmed evidence of adenoviral infection in patients with appendicitis. Furthermore, qPCR is as useful and reliable as IHC in diagnosing adenovirus in appendicitis, showing higher sensitivity compared to IHC.

Author contributions statement

LHL carried out experiments, performed statistical analysis and visualization and wrote the original draft of the paper. CJH conceived and designed the study, reviewed and revised the paper and provided material support. CYL and YHL provided material support, curated data, and reviewed and revised the paper. All authors were involved in writing the paper and had final approval of the submitted and published versions

Ethics approval

This study was approved by the institutional review board (IRB) of the Cathay General Hospital, Taipei, Taiwan, approval number CGH-P110068.

The entire research has been performed in accordance with the Declaration of Helsinki to fully respect patients' privacy.

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Conflicts of interest and source of funding

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Competing Interests

The authors have no relevant financial interest in the products or companies described in this article.

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