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A Case of Bacillus Calmette-Guérin Cystitis Diagnosed with a Novel Loop-mediated Isothermal Amplification Method

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ABSTRACT

Intravesical bacillus Calmette–Guérin (BCG) instillation is broadly used to prevent bladder cancer recurrence or to treat carcinoma in situ. BCG infection is rare but can cause serious problems because this strain has intrinsic resistance to pyrazinamide, a first-line anti-tuberculosis drug. Furthermore, there had been no specific and easy procedure accurately diagnosing BCG infection. In this case report we present the first case of BCG cystitis diagnosed with a newly developed easy-to-use diagnostic procedure using the loop-mediated isothermal amplification method.

Introduction

Bacillus Calmette-Guérin (BCG) has intrinsic resistance to pyrazinamide, a first-line anti-tuberculosis drug.[1] Therefore, when bladder tuberculosis is suspected, it is crucial to distinguish BCG from other Mycobacterium tuberculosis complex (MtbC) subspecies, especially in patients who previously received BCG instillation.

Classical standard procedures based on conventional culture and characterization of biochemical features are time-consuming and cannot distinguish BCG from MtbC subspecies. The conventional loop-mediated isothermal amplification (LAMP) method is the fastest and most convenient way to detect MtbC in clinical settings, but it cannot distinguish BCG from MtbC subspecies. A polymerase chain reaction (PCR) test targeting the region of difference 1 (RD1),[2] which is missing from all BCG strains, can be used to distinguish BCG from other MtbCs subspecies but is not commonly used in clinical settings.

We report herein the first case of BCG cystitis diagnosed with a newly developed easy-to-use LAMP method[3] designed to detect the lack of RD1, that is, to amplify the BCG gene specifically.

Case presentation

A 76-year-old male patient underwent transurethral resection of bladder tumor and was pathologically diagnosed with pT1 urothelial carcinoma with carcinoma in situ. He was then given 80 mg of intravesical BCG (Tokyo 172 strain) once a week for 8 weeks. After the treatment, he received regular cystoscopy and urine cytological examinations. Two years after the treatment he complained of pain on urination, urinary frequency, and gross hematuria. Urine cytology was negative for cancer cells and urine culture revealed no urinary tract infection by general bacteria. Cystoscopy showed reddish erosive mucosa covering the entire bladder (Fig. 1). Bladder tuberculosis was suspected and urine culture for mycobacterium demonstrated the presence of MtbC.

To distinguish BCG from other MtbC subspecies, we used a newly developed LAMP method that can detect BCG in urine. This method uses an oligonucleotide LAMP primer designed to detect the lack of the RD1 sequence and amplifies the BCG gene specifically.[3]

A clear color change from pale brown to cloudy yellow-green was observed under natural light, demonstrating the successful amplification of the BCG gene and therefore the presence of BCG in the sample (Fig. 2A). The change in sample turbidity also confirmed the successful amplification of the BCG gene (Fig. 2B, increased turbidity means amplification of the BCG gene).

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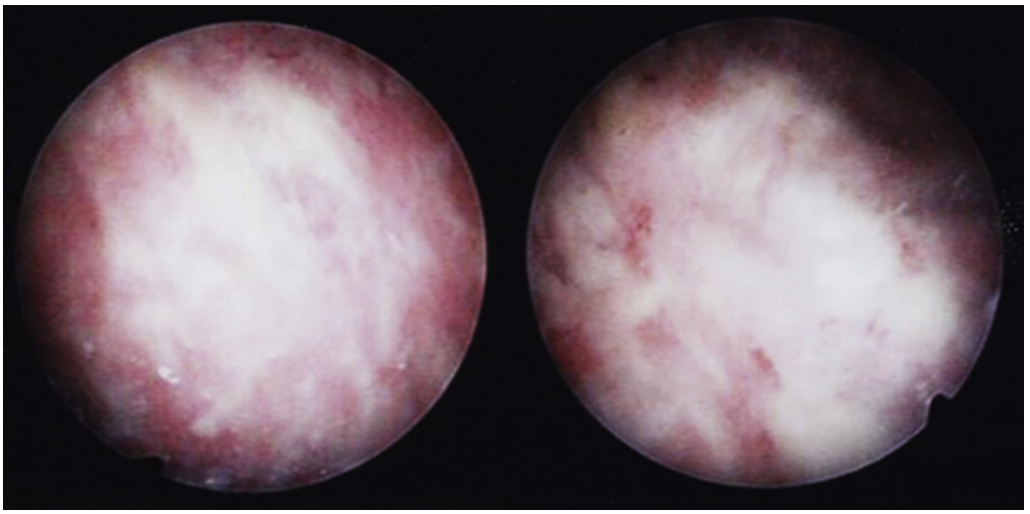

Figure 1. Cystoscopy at presentation. Reddish erosive mucosa covered the entire bladder.

To evaluate the diagnostic accuracy of the present LAMP method, we also performed a PCR test for RD1 deletion using the patient's urine sample prior to anti-mycobacterium chemotherapy. This test confirmed that the MtbC isolated from the patient's urine was BCG rather than some other MtbC subspecies (Fig. 2C).

The patient was then treated with anti-mycobacterium chemotherapy consisting of isoniazid and rifampicin, and his bladder irritation was alleviated in two weeks. The follow-up LAMP test 30 days after the initiation of anti-mycobacterium chemotherapy showed positive results and the results became negative thereafter (Fig. 3).

Discussion

The incidence and severity of adverse effects following intravesical BCG instillation depend on a number of factors, such as the dosage, the recipient's age, and the BCG strain.[4] Whereas systemic

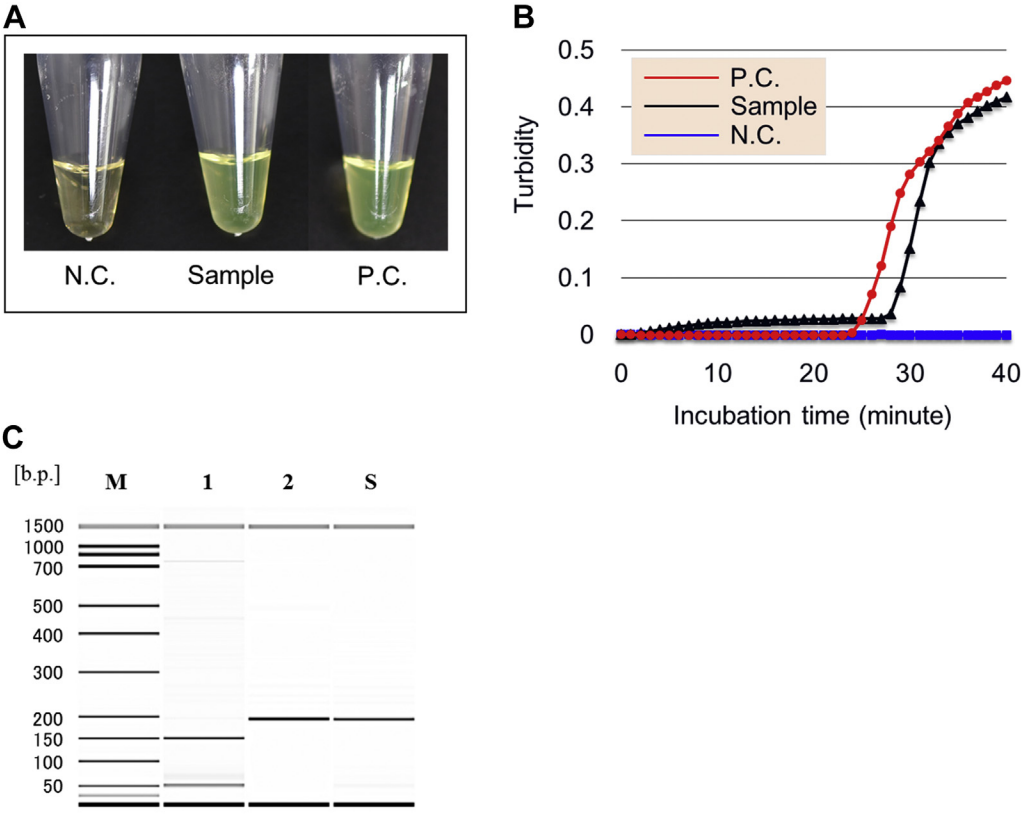

Figure 2. (A) Detection of bacillus Calmette-Guérin (BCG) in the patient's urine sample by the loop-mediated isothermal amplification method. Color change from pale brown to cloudy yellow-green means successful amplification of the BCG gene. N.C., negative control; P.C., positive control. (B) Time-dependent change in the turbidity of the samples. Increased turbidity means successful amplification of the BCG gene. (C) Polymerase chain reaction test for region of difference 1 (RD1) absence confirmed that the MtbC isolated from the patient's urine was BCG rather than some other MtbC subspecies. b.p., base pair; M, lane marker; 1, *Mycobacterium tuberculosis*; 2, BCG; S, patient's urine sample.

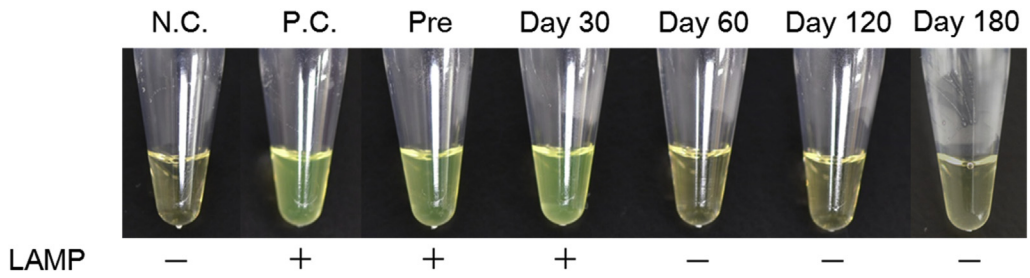

Figure 3. Loop-mediated isothermal amplification (LAMP) method results showing the changes in bacillus Calmette-Guérin gene amplification after anti-mycobacterium chemotherapy. N.C., negative control; P.C., positive control; Pre, sample obtained before treatment.

complications are usually observed within 3 months after BCG instillation, focal complications might occur >1 year after the instillation.[5] BCG cystitis should therefore be considered if cystitis-like symptoms are observed long after the completion of BCG instillation. Because most BCG strains have intrinsic resistance to pyrazinamide, a first-line anti-tuberculosis drug,[1] differentiating BCG from other MtbC subspecies is vital when MtbC is detected in a patient's urine specimen.

Nucleic acid amplification by the newly developed LAMP method needs only a heating block to amplify the specific target gene and takes only 60 minutes to complete.[3] In the present case it detected BCG as accurately as PCR did and was thus demonstrated to be useful for detecting BCG in clinical settings faster and more easily. It also facilitates our on-going clinical study investigating how BCG residual rates change over time after BCG instillation, which had required the use of more complex and time-consuming procedures.

Conclusion

We reported a case of BCG cystitis diagnosed with a novel LAMP method detecting BCG. This is the first report of the clinical application of this diagnostic method.

Consent

Written informed consent was obtained from the patient for publication of this case report.

Conflict of interest

We have no conflict of interest to declare.

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