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Original Article

Detection of Disseminated Mycobactrial Infection Due to BCG Vaccination (BCGosis) in 4 Suspected Infant Autopsies by PCR Method

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ABSTRACT

Background and Objective: **BCG vaccination is used in many countries with a high prevalence of TB to prevent childhood tuberculosis meningitis and miliary disease. Local and systemic side-effects are associated with BCG vaccine. The most critical reaction is disseminated BCG infection which occurs in mostly immunodeficient patients.**

Materials and Methods: **We performed 4 autopsies during 2001-2003 which were suspected for BCGosis clinically and histologically by presence of granulomatous foci in several organs with acid fast bacilli. The mycobacteria were identified by PCR. Their DNA was extracted from the tissue blocks, identified with primers which were designed to detect the RD1 deletion.**

Results: **We found BCG genome by PCR in 3 out of 4 patients. These patients had acid fast bacilli in special staining.**

Conclusion: **Since BCGosis is a fatal and uncommon disease, occurring after vaccination with numerous complications, its diagnosis is of paramount importance and should be considered in the appropriate clinical setting.**

Key words*:* Granuloma, Autopsy, PCR

Introduction

BCG is a live attenuated strain of *Mycobacterium bovis* that was first used for immunization against tuberculosis in 1921. Over 3 billion doses of BCG vaccine have been given since 1948, and it has been considered safe (1). Iranian vaccination protocol employs vaccination of BCG against tuberculosis at birth (2).

Clinical recognition of BCGosis in immunocopromised patients is difficult but it should be considered in cases with unexplained febrile illness, functional abnormalities in multiple organ systems, and a history of vaccination with BCG (3-5). Therefore, the ability of rapid and specific identification of BCGosis in these patients is clinically critical.

The conventional detection of mycobacteria in clinical samples is based on the demonstration of the acid fast organisms followed by culture. This method is reliable but it is time-consuming and also formalin liable. The growth of the organisms can take at least 6 to 8 weeks, it is limited by the low level of sensitivity and specificity and it does not allow for the detection of mycobacteria at concentrations below 104/ml. Amplification of specimen target DNA by PCR, which allows it to be detected in low levels of concentration, helps us in early detection and better identification of the source of infection (6,7).

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One region difference, designated RD1 was found to be present in all virulent *M. bovis* and *M. tuberculosis* strains but deleted from all BCG which vaccine strains tested. Talbot et al. designed primers to amplify the complement sequence of RD1 with this information. A PCR method was developed to detect the RD1 deletion. This method can be used as a tool for rapid and specific identification. BCG will show 200 bp bands as a deletion of RD1 region (7).

Therefore, we examined 4 autopsy cases between 6 months to one year old with a history of BCG vaccination and clinical picture of fulminant sepsis or malignancy from 2001 to 2003 where necrotizing granulomas with acid fast bacilli were present in various organs. We were encouraged to determine the presence of BCG genome as the causative agent in such patients by PCR method. This descriptive cross-sectional study can be helpful in determining patients with BCGosis which is defined as disseminated BCG infection overriding on immunodeficient patients with a background of IL6, IL12, and IFNγ deficiency (8).

Materials and Methods

Patients and specimens

This study was performed in Pediatric Infections Research Center (Mofid children hospital). Eleven tissue blocks belonging to four dead infants and newborns who were admitted for severely progressive systemic disease with clinical diagnosis of sepsis and a history of BCG vaccination between 2001 and 2003 were selected for this study (Table1). These autopsies had been done under approval of the parents and blocks had been taken from specific organs suspicious for BCGosis determined previously by histological routine H & E assessment with presence of many necrotizing granulomata. All of them were positive for acid fast bacilli. One of the 4 cases was associated with an immune deficiency of B&T cells.

DNA extraction

For this purpose, 20 µm sections of each paraffin-embedded tissue blocks were dewaxed twice with 1 ml of xylene for 5 min and centrifuged (10000 rpm, 5 min). The supernatant was discarded and traces of solvent were removed by washing the pellet twice for 5 min with 1 ml of 100% ethanol. After centrifugation, the pellet was air dried (9-11). DNA was purified using the Qiamp mini kit (Cat. No. 51306; Qiagen).

PCR amplification

PCR amplification was performed using 25 µl Taq PCR master mix (Cat. No. 201443; Qiagen) containing 2.5 units of Taq DNA polymerase, 1 x mgcl2 and 200 µm of each dNTP and 5 pmol of primers ET1: 5'-GCGGTTGCCGC CGACCGACC GACC-3' and ET3: 5'-GAGGCGATCTGGCGGTTTGGGG-3'(7). Then, 5 µl of each coded DNA sample in a total volume of 50 of 20 µl of PCR mix. The mixtures was denatured for 3 min at 95°C and cycled 40 times to 94°C for 30 seconds and 65°C for 1 min, followed by a final 10 min extension at 72°C. The positive control was *mycobacterium bovis* (BCG from Pasteur institute) and the negative control was distilled water. PCR products (200 bp) were separated by electrophoresis on a 3% Agarose gel in Tris-Boric acid-EDTA buffer. Presence of each PCR product was determined by UV transillumination of the ethidum bromide stained gel (7, 9).

Results

Totally, 11 paraffin-embedded tissues from different organs belonging to 4 dead patients less than one year old had been selected from Mofid Children hospital, Department of Pathology, during the years 2001-2003. The socioeconomic status, clinical and paraclinical characters, and histologic data related to BCGosis are shown in the Tables 1-3.

Table 1. Socioeconomic status of 4 suspected patients admitted for BCGosis during 2001-2003

patient	Age (months)	gender	Birth order	Presence of previously expired siblings	Consumption of cow milk	Father 's job	Mother's job	Living place
1	8	F	3	-	+	Worker	Housekeeper	urban
2	3	F	4	-	-	Worker	Housekeeper	urban
3	8	M	2	1	-	Farmer	Housekeeper	Suburban
4	5	F	2	-	-	Farmer	Housekeeper	Urban

Table 2. Clinical characters of 4 suspicious patients for BCGosis from 2001 to 2003

Patient	Weight	Height	Head circumference	A	H	S	Dermal lesions	Lesions in lungs	Lesions in abdomen	CNS lesions	History of Immunodeficiency
1	5	64	40	+	+	+	+	+	+	-	-
2	3.9	59	34	+	+	+	+	+	+	-	-
3	8	70	43	-	+	+	-	+	+	+	+ (B&T cell)
4	7.3	62	43	-	+	+	-	-	+	-	-

(A) Adenopathy, (H) Hepatomegaly, (S) Splenomegaly

Table 3. Paraclinical data of 4 suspected patients admitted for BCGosis during 2001-2003

Patient	Hb	ESR	WBC	Neutrophil (%)	Lymphocyte (%)	CRP
1	6.4	36	3200	35	70	+
2	7	114	14500	55	40	++
3	10	105	14000	60	25	+++
4	10.4	110	5000	60	30	++

All patients had been admitted mostly for severe acute respiratory distress and fever following BCG vaccination. They had necrosis with granuloma in one or more organs. Acid fast bacilli were detected in all complicated organs. One of these patients (patient number 2) had diffuse bilateral myofibromatosis of both lungs in addition to identifying mycobacterium bacilli in all organs, and the other patient (patient number 3) with positively detected BCG genome had a history of B cell and T cell deficiency according to flowcytometry report.

BCG genomes were detected in 9 out of 11 paraffin-embedded blocks belonging to 3 patients by PCR method (Fig. 1). These positive results were more frequent in females. Also, the age of patients with two positive samples was 5 and 8 months (Table 1). One of them was admitted in 2003 and the other one in 2001 (Table 4).

Histologically, necrosis and granuloma formation were identified in different organs in all PCR positive cases. The frequency of presence of granulomata and necrosis in involved organs and the correlation with PCR positivity has been shown in Table 4.

Table 4. Detection of BCG genome in different organs in 4 suspected autopsies

patient	Age (months)	Date of sampling	Histological features	Involved organs	Acid Fast Bacilli	PCR
1	8	2003	Granulomata	Spleen, right lung	+	+
2	3	2001	Necrotizing granulomata	Liver, lung	+	+
3	8	2003	Necrotizing granulomata	Left and right lung, spleen, liver, lymph	+	+
4	5	2001	Granulomata	Liver, spleen	+	-

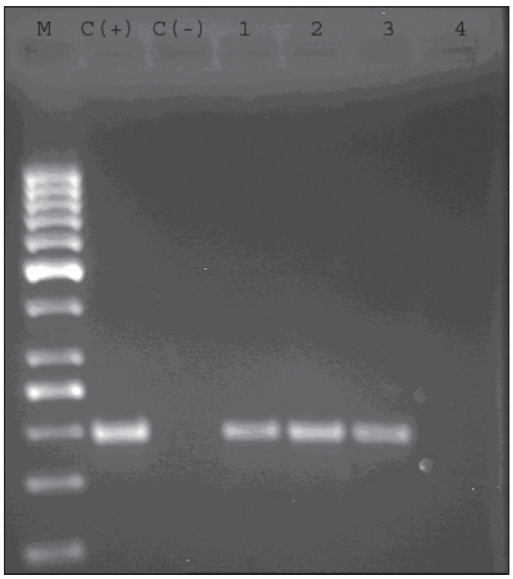

Figure 1. Agarose gel electrophoresis of the PCR products (200bp). PCR products of M. bovis BCG (Pasteur institute) (Lane 2), Negative sample (lane 3) and selected positive samples (lanes 4-6) in 3 BCGosis patients

Discussion

The attenuated bacillus Calmette-Guerin (BCG) vaccine is administered to prevent tuberculosis. Complications of vaccination are uncommon (12). The most important and critical adverse reaction of BCG vaccination is disseminated BCGosis (3, 4). Disseminated BCG has historically been a disease of infants, but cases now occur in adults and older children co-infected with human immunodeficiency virus. Such cases also occur after revaccination of individuals who were anergic following the initial administration of BCG vaccine (12).

In the current study we detected BCG genome by PCR in 3 out of 4 suspected patients with clinically progressive sepsis. All of these patients had high grade fever several weeks or months after BCG vaccination with multi organ involvement including liver, kidneys, GI, bone (rib), CNS, and etc. On the other hand, necrosis with granulomas and acid fast bacilli have been identified in autopsies of all 4 patients in several organs.

Culture is considered to be the gold standard for detecting Mycobacteria, but this is a very slow and labor intensive procedure (13). PCR-based methods are useful for rapid detection of target DNA in suspected clinical samples. The efficacy of PCR assays can be influenced by the quality of target DNA extracted from appropriate samples (14). Therefore to identify the incidence of complications and virulence among BCG vaccinated patients, applying the PCR method might be effective (11).

We did this project to we confirm our histologic and clinical diagnosis of BCGosis with PCR on tissue paraffin-embedded blocks to see whether it is helpful or not?

RD1 segment is not present in all *M. bovis* BCG strains, but it is present in other strains of the *M. tuberculosis* complex. For *M. bovis* BCG strains, Talbot et al. designed primers to amplify the complement sequence of RD1 (7, 14).

In this study, BCG genome was detected in 3 out of 4 tissue samples and the deleted segment was not identified in the other negative specimens. An et al concluded due to the presence of inhibitors like formalin in fixation procedure, tissue processing, and deparaffinization and other endogenous unknown inhibitors, there can be false negative results (15). Also, involvement by other members of the *M. tuberculosis* complex (*M. avium, and M. avium subsp*.) may be particularly the cause of a negative PCR result. Juana Magdalena et al detected BCG genome and also the other type of mycobacterium complex family in 148 patients' tissue samples of paraffin blocks (16).

Conclusion

Disseminated BCG disease is an uncommon but devastating complication of vaccination that should be considered in the appropriate clinical setting and it is better to be aware of the patient's family history before applying BCG vaccination, especially in immunocompromised patients at birth and also in patients with late-stage AIDS who are at greatest risk and respond poorly to standard therapies.

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