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Case Report

Clinical and laboratory observation of Bacillus Calmette-Guérin infections

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Abstract: Objective: To study the clinical features of BCG infection in children. Methods: 51 cases confirmed with BCG infection from all over China were enrolled and followed up for at least 6 months. All cases were treated with anti-tuberculosis drugs. A random, open, group control study was designed in non-disseminated cases to evaluate curative effects of anti-tuberculosis drugs for early stage BCG infection. Disseminated cases were also closely monitored, and patients were given combined anti-tuberculosis drug therapy. Results: In 34 (66.7%) non-disseminated cases, 19 children with local infections were treated with Isoniazid (Group A) and 15 were treated with Isoniazid and Rifampin (Group B). In the first 3 months, Group B responded better to anti- tuberculosis drug therapy than Group A ($P<0.05$). At the end of 6 months drug therapy, improvement rate was 100% of Group B vs. 89.5% of Group A ($P<0.05$). 33.3% children were admitted with disseminated BCG disease and were initially treated with Isoniazid and Rifampin. Most of these children responded poorly to drug therapies: Both isolated strains and BCG vaccination strain showed resistance to isoniazid, but susceptible to other First-line anti-tuberculosis drugs (Rifampin, Ethambutol and Streptomycin). Conclusion: INH does not perform well for treating BCG Chinese infections. Multiple drug regimens are necessary for treatment and preventing Drug-Resistance. Even for non-disseminated cases, preventive therapy using mono-isoniazid regimen is not suitable. BCG infections also occur in children without clear immunodeficiency, so parental education and awareness of health-care workers is essential for promptly recognition and handling BCG infections.

Keywords: BCG, disseminated BCG disease, therapy, drug susceptibility test

Introduction

For high-burden countries, BCG vaccination is an important strategy preventing the spread of tuberculosis. WHO recommends vaccination as soon as possible after birth. At present, BCG is administered to all the newborns in China. As is known, BCG vaccine is a live attenuated vaccine derived from *Mycobacterium bovis* [1, 2]. Local adverse reactions, including erythema, induration or small ulceration around injection, are relatively common, and these reactions disappear in several weeks. Systemic adverse reactions are rare but increasingly reported in recent years [3-5]. Before 2009, we accepted some children patients with adverse reactions after BCG vaccination. We observed the cases and found that those with minor local adverse reactions cured by themselves, but some with cutaneous abscesses or lymphadenopathies stayed unrecovered until medication. Considering drug safety, only few kinds of drug can be used in children for treating BCG infections. Studies [6, 7] proved that *Mycobacterium bovis* is intrinsically resistant to Pyrazinamide (PZA). Therefore, Isoniazid (INH) and Rifampin (RFP) seem to be pivotal choice for BCG infctions. With Anti-TB drug treatment, we also saw few children with BCG infections died or responded very poorly, which confused us for quite a period. In 2009, we embarked on a systematic case collection and observation on children TB in Shanghai Public Health Clinical Center. The objective of this study is to see the clinical features and provide the basis for medication to BCG infection in children

Methods

Data sources

Between the year 2009-2012, we accepted 174 TB cases <18 months with BCG vaccination history from all parts of China, in which 51

children were confirmed with BCG strain infection by tissue bacterial culture and identification. No one had accepted systematic anti-tuberculosis treatment. The characteristics, symptoms, immune level, treatment results and susceptibilities of isolated strains were analyzed. This study was approved by Ethics Committee of Shanghai Public Health Clinical Center.

Diagnostic criteria

BCG infection is proven by following condition: (i) definite history of BCG vaccination followed with local cutaneous abscesses or lymphadenopathy; (ii) strains proven M. bovis BCG strain by polymerase chain reaction (PCR) analysis. Disseminated BCG disease is defined as those with histopathological demonstration of acid-fast bacilli at two or more anatomic sites far from the region of vaccination such as lymph nodes or cutaneous abscesses outside the region of inoculation, liver biopsy, lung biopsy, cerebrospinal fluid, gastric aspiration and bone marrow aspiration [1, 3].

Strain culture, identification and drug susceptibility tests

Isolated strains were cultured by Bactec MGIT 960 system according to the manufacturer's recommendations. Drug resistance was defined as when there was greater than 1% growth of mycobacterial strains in the presence of 0.1 μg/ml of INH, 1.0 μg/ml of RFP, 5.0 μg/ml of EMB and 1.0 μg/ml of streptomycin (S), respectively. Susceptible means no visible growth of a mycobacterium after culture. Minimum inhibitory concentration (MIC) was defined as the lowest concentration of an anti-tuberculosis drug that will inhibit the visible growth of a mycobacterium after culture by Bactec MGIT 960 system. Region of Difference 1 (RD1), a genomic region which was found to be present in all virulent *M. bovis* and *M. tuberculosis* strains but deleted from all BCG strains was tested by PCR assay to identify BCG strain from *M.tuberculosis* [7, 8]. Drug susceptibility of isolated strains and BCG Chinese strain and *M. tuberculosis H37Rv* strain were determined using Bactec MGIT 960 system at the recommended concentrations [9, 10].

Immune level evaluation

BCG disease has been reported in primary immunodeficiency such as chronic granulomatous disease (CGD) and severe combined immunodeficiency (SCID, especially IL-12/IFN-γ axis defect for BCG infection), [1, 11]. The competency of the immune system was evaluated by different tests including measurement of immunoglobulin levels, neutrophil granulocyte and phagocyte activity, T cell and B cell flow cytometry, and HIV ELISA test. Neutrophil granulocyte and phagocyte activity was tested by the flow cytometry assay and neutrophil respiratory burst test using the DHR assay (Phagocytes swallow Dihydrorhodamine 123 and shine).

Grouping and treatment

Non-disseminated cases were divided into two groups randomly. One group had INH preventive therapy (Group A) and another treated with INH and RFP (Group B). For disseminated cases, INH and RFP were given as initial treatment. Superficial suppurations were aspirated to enhance recovery and smeared to monitor the efficacy of treatments.

Statistical analysis

Categorical variables were calculated as percentages. Treatment outcomes were compared between Group A and Group B with the use of Fisher's exact test.

Results

Characteristics of the patients

51 children confirmed with BCG strains infections involved in this study (**Table 1**). Male sex takes the majority of the patients (74.5%). The median onset age was 3 months (interquartile range, 2 to 4). Common symptoms include lymphadenopathy (92.2%), hepatosplenomegaly (55.0%), Fever >37.5°C (27.5%) and Local cutaneous abscesses (21.6%). Fever and hepatosplenomegaly were more common in disseminated BCG infections. 34 (66.7%, 34/51) children were non-disseminated cases, 19 given INH (Group A) treatment and 15 (Group B) given INH+RFP treatment for at least 6 months. Another 17 children were disseminated cases, in which only 3 finished 6 months INH+RFP treatment. The other disseminated children developed severe complications and were treated with multiple anti-tuberculosis drugs according to their conditions.

Table 1. Characteristics of 51 children infected with Bacillus Calmette-Guérin strains

Characteristic	Value			
	Group A	*Group B*	*Disseminated*	*Total*
	19	15	17	51
Male Sex	14	12	12	38 (74.5)
Onset Time				
<1 month	2	1	0	3 (5.9)
1-3 month(s)	11	11	11	33 (64.7)
3-6 months	4	3	4	11 (21.6)
6-9 months	2	0	1	3 (5.9)
9-12 months	0	0	1	1 (2.0)
>12 months	0	0	0	0(0)
Signs or symptoms-number (%)				
Lymphadenopathy	18	13	16	47 (92.2)
HSM	8	6	14	28 (55.0)
Local cutaneous abscess	5	2	4	11 (21.6)
Fever >37.5°C	2	1	11	14 (27.5)
Immunodeficiency Disorder- number (%)				
SCID	0	0	2	2 (4.0)
CGD	0	0	1	1 (2.0)
CVID	0	0	1	1 (2.0)
Achondroplasia	0	0	1	1 (2.0)

Footnotes: HSM, hepatosplenomegaly; SCID: severe combined immunodeficiency; CGD: chronic granulomatous disease; CVID, common variable immunodeficiency.

Table 2. Drug susceptibility test of different strains to HRES*

	Isoniazid (0.1 μg/ml)	Rifampin (1.0 μg/ml)	Ethambutol (5.0 μg/ml)	Streptomycin (1.0 μg/ml)
Isolated strain	R**	S**	S	S
BCG Chinese strain	R	S	S	S
M. Tuberculosis H37Rv	S	S	S	S

Footnotes: *Concentrations labeled under different drugs are Critical Concentrations worldwide used in M. tuberculosis drugs susceptibility testing by Bactec MGIT 960 system. **S, susceptible; R, resistant.

Treatment outcomes

In non-disseminated cases: After 3 months treatment, the improvement rate of Group A was 73.7% (14/19). 14 children had bacteriological and radiological improvement, but 5 still acid-fast bacilli positive at lymph nodes aspiration or cutaneous abscesses. Group B had better response to drugs, and the improvement rate was 93.3% (14/15), better than Group A, $P<0.05$. One was acid-fast bacilli positive after 3 months treatment. At 6 months end, the improvement rate of Group A and Group B were 89.5% (17/19) and 100% (15/15), $P<0.05$. No one developed to disseminated during 6 months treatment. Drug adverse reactions, including one mild abnormal liver function and one drug hypersensitivity (erythema) found in Group A, three mild abnormal liver function in Group B, no significant difference.

In disseminated cases: All 17 children responded poorly to drug therapies, 3 died, 1 developed into MDR-TB. The symptoms improved slowly, and acid-fast bacilli of lymph nodes aspiration or gastric aspiration kept positive. Lesions progressed in 12 children, and they changed to multiple anti-tuberculosis drugs including Ethambutol, Amikacin, or Linezolid.

Laboratory findings

Drug susceptibility tests (DST) of isolated strains, *BCG Chinese* strain and *M. tuberculosis H37Rv* were carried out by Bactec MGIT 960 system. All strains were susceptible to Rifampin, Ethambutol and Streptomycin. All isolated strains and BCG Chinese strain were tested resistant to INH. MICs of isolated strains and *BCG Chinese* strain were 0.4 μg/ml

Table 3. Data of 5 disseminated cases with immunodeficiency

	Sex	Onset Time (months)	Symptoms	Immunodeficiency	Drug treatment	Outcome
1	female	3	Lymphadenopathies beyond injection, lung lesions	SCID* with severe anemia	INH, RFP, EMB, Amikacin	Unrecovered
2	male	3	lymphadenopathy, cutaneous abscesses, lung and bone lesions	SCID* with extreme high blood immunoglobulin E levels (over 100000ug/L)	INH, RFP, EMB, Amikacin, Linezolid	Died
3	male	4	lymphadenopathy, lung and bone lesions	Congenital achondroplasia combined with low level of T cells (CD4 count drops below 100, HIV negative)	INH, RFP, EMB, Linezolid	Died
4	male	3	lymphadenopathy, cutaneous abscesses, lung lesions, bone lesions	CGD**	INH, RFP, EMB, Linezolid	Unrecovered
5	male	12	Lymphadenopathies beyond injection, lung lesions	CVID***	INH, RFP, EMB, Amikacin	Died

Footnotes: *low level of phagocyte activity, mutation of IL-12 R beta 1 gene sequences tested by PCR assay; **low level of phagocyte activity, mutation of CYBB; ***High immunoglobulin M syndrome.

(**Table 2**). After immunological tests, 5 children (29.4%, 5/17) in disseminated cases were found with congenital or acquired immunodeficiency disorders including abnormal immunoglobulin level, low phagocyte activity, low CD4+ cell counts or B cell counts. Their gene sequences were tested by PCR assay, and 2 confirmed SCID, 1 CVID, 1 CGD (**Table 3**). None immunodeficiency disorder was found in non-disseminated cases.

Discussion

Mycobacterium bacillus Bacillus Calmette-Guérin is an attenuated strain derived from an *M. bovis* isolate in the early 20th century which was distributed worldwide as an important immunization strategy against tuberculosis. As the genes transferring, BCG sub-strains showed a certain difference in molecular genetic characteristics with each other. Some sub-strains, such as *BCG-Pasteur 1173* and *BCG-Denmark 1331*, are relativity stronger and more virulent [4]. In China, BCG strains currently in active were derived from *BCG-Denmark 823* and the coverage is estimated to be over 90% [12]. Although BCG vaccine for tuberculosis has an outstanding safety record, safety problems deserves sustained attention. In the past years, children BCG infection was underestimated, and severe adverse reactions due to BCG injection were rarely reported in China. In most undeveloped regions of China, strain identification is really hard for clinical laboratories. BCG lymphadenopathies were often misdiagnosed as tuberculosis lymphtitis or non-specific lymphtitis and disseminated BCG disease were often diagnosed as severe tuberculosis or bacterial infections. In 2009-2012, 174 TB cases <18 months with BCG vaccination history throughout the country were hospitalized in Shanghai Public Health Clinical Center and 51 of them (28.7%) were confirmed with BCG infection. Only 5 disseminated BCG infection cases had clear immunodeficiency disorders. No clear immunodeficiency-disorder evidence in another 46 children. This implies that BCG vaccination has potential risks for all newborns including those without immunodeficiency problems.

For all involved cases, obvious symptoms of BCG infection usually appeared in the first 3 months after vaccination (70.6%). Over 90% cases got the first symptoms before 6 months old. Lymphadenopathy was most common reason (92.2%) for hospitalization. Hepatosplenomegaly was also common (55.0%), but not easy to be noticed by parents. Fever >37.5°C appeared in 64.7% disseminated cases, but only 8.8% for non-disseminated. Local cutaneous abscess is often a mild adverse reaction due to BCG injection, and most single cutaneous abscesses recover by dressing changes. That is why local cutaneous abscess was uncommon in this study.

BCG infections usually need medication. Referenced to the treatment of *M. tuberculosis* infection, the first-line anti-tuberculosis drugs were considerable for children. INH, RFP, PZA are most important drugs for *M. tuberculosis H37Rv* infection but not suitable for BCG infection. *Mycobacterium bovis* is intrinsically resistant to PZA [9] and less sensitive to INH than *M. tuberculosis H37Rv*. Reported MIC of isoniazid for *M. tuberculosis H37Rv* was 0.05 μg/ml [13], however, the MIC for *BCG Chinese* strain we tested was 0.4 μg/ml. Although serum concentrations in children given a dose of 10 mg/kg of body weight isoniazid exceed the MIC of 0.4 μg/ml [14], this kind of "resistant" introduced uncertainty into treatment outcomes. For non-disseminated cases, nono-INH regimen is not as good as INH+RFP regimen. Two drugs regimen showed high cure rate with acceptable complications. We suggest it for local BCG infections.

In disseminated cases, systemic symptoms are more common and patients have higher risk of further dissemination. Therefore, at least 4 effective drugs (we suggest including RFP, EMB and aminoglycoside) are needed. INH may not be used as a key drug for disseminated BCG infectons. Given proper drug treatment, immune status are critical prognostic factors for disseminated BCG infection. Patients with congenital immunodeficiency are significantly more difficult to recover and require a much longer period of treatment.

In this study, we noticed that one disseminated case turned to MDR-TB (resistant to INH and RFP) who was treated with INH+RFP initially. The first DST (Bactec MGIT 960 system) showed INH resistant, but RFP susceptible. After 3 months treatment, the patient had no sign of improving, and the following DST showed both INH and RFP resistant. This mutation may result from high bacteria load and the "less

susceptible" to INH. Since INH is weakened for BCG strain, INH+RFP regimen is not definitely safe to prevent the occurrence of drug resistance, especially in disseminated BCG infection, which spread much more bacteria in the body.

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Disclosure of conflict of interest

None.

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