Post-Vaccination Disseminated Bacillus Calmette Guerin Infection Among Children in Southern Iran

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Abstract

Background: Disseminated bacillus calmette guerin (BCG) infection is a rare but life threatening complication of BCG vaccination. It has been mainly seen in severe immune deficiency. A precise and rapid diagnosis is crucial for prompt initiation of an aggressive antimycobacterial treatment. Polymerase chain reaction (PCR) is directly applicable to smear-positive clinical specimens, proven to be a rapid and specific diagnostic test.

Objectives: The aim of this study was to investigate disseminated BCG infection among 34 children in southern Iran, mainly confirmed by PCR.

Patients and Methods: We included all the patients hospitalized with disseminated BCG infection at a referral teaching hospital in southern Iran between years 1990 and 2007. The clinical and laboratory data including the immunological workups were obtained through a review of the medical files. We recalled all pathology samples from pathology specimen banks and used an in-house PCR specific for Mycobacterium bovis BCG substrain to confirm the diagnosis.

Results: From the total of 34 children hospitalized with disseminated BCG infection, 21 were categorized as definite and 13 probable. Thirty-one patients (91%) were under two years of age and 41% were male. The most common clinical findings were fever in 31 (91.2%), axillary’s lymphadenopathy in 26 (76.5%), hepatosplenomegaly in 25 (73.5%), stunted growth in 21 (61.8%), and distant lymphadenopathy in 16 (47.1%). Polymerase Chain Reaction positivity rate was 100% (9 of 9) in bone marrow smear slides and 84.2% (16 of 19) for formalin-fixed and paraffin-embedded tissue specimens. Immunodeficiency state was detected in 50% and the overall mortality rate was 58.8% (20 of 34).

Conclusions: Disseminated BCG infection should be considered in the differential diagnosis of infants and young children with fever, hepatosplenomegaly, lymphadenopathy, and history of BCG vaccination. The PCR method has a high positivity rate and can serve as a useful tool for the rapid and specific identification of M. bovis BCG substrain infection.

Keywords: BCG Vaccine, Disseminated BCG and Salmonella Infection, Immunologic Deficiency Syndromes, Polymerase Chain Reaction, Iran

1. Background

Bacillus calmette guerin (BCG) vaccine, derived from multiple passages of wild-type Mycobacterium bovis, has been used in humans since 1921 and is still the only vaccine available against tuberculosis (1). It has a relatively high protective efficacy against meningeval and miliary tuberculosis. Approximately, 100 million children routinely receive one or more BCG vaccines in most countries of the world every year and in Iran yearly, 1.4 million neonates receive one dose at birth as per the expanded immunization program (2). Although BCG immunization is relatively safe, it may be associated with adverse events, such as self-limited injection site lesions, regional adenitis, and in rare cases, life threatening disseminated BCG infection (3). The incidence of disseminated BCG infection has previously been reported to be 0.19 - 1.56 cases per one million vaccinated infants and such disease has been mainly seen in severe immune deficiency, with high mortality rate (3, 4).

Diagnosing disseminated BCG infection that affects mainly infants and young children and is characterized by fever, weight loss, lymphadenopathy, and hepatosplenomegaly requires a high index of physicians’ suspicion (5). A wide variety of differential diagnosis should be considered including other mycobacterial,
some bacterial, viral, parasitic, and fungal infections as well as neoplastic and connective tissue diseases, and also metabolic or hematologic diseases with superimposed infections (3, 5). To confirm the diagnosis, differentiation of isolated *Mycobacterium* BCG sub-strain from atypical mycobacteria and other members of *Mycobacterium tuberculosis* complex (MTC) such as *M. tuberculosis*, wild-type *M. bovis*, and *M. africanum* by various methods is mandatory (5). Although isolation by culture and then identification are the optimal confirmatory steps in diagnosis, Polymerase chain reaction (PCR) is directly applicable for smear-positive clinical specimens, indicating that it is a rapid and specific diagnostic test. A precise and rapid diagnosis of disseminated BCG infection is crucial in starting a prompt aggressive anti-mycobacterial treatment, which needs to be continued for a prolonged period of time (3, 5).

2. Objectives

The present study aimed to report on the various manifestations, laboratory findings and outcomes of this rare life-threatening complication in a considerable number of infants and young children managed at a single center and to propose PCR as an efficient confirmatory diagnostic method applied directly on pathologic specimens.

3. Patients and Methods

We retrospectively reviewed the medical files of all children hospitalized for disseminated BCG infection at Nemazee Teaching Hospital, a referral hospital in southern Iran, between 1990 and 2007. All of the 34 individuals included in this study were diagnosed and treated as disseminated BCG infection, by a pediatric infectious diseases specialist. The presumptive diagnosis was based on (a) the presence of systemic syndromes compatible with disseminated mycobacterial disease, (b) a positive history of BCG vaccination, (c) a temporal relationship between BCG vaccination and disease, (d) the presence of disease in the region of vaccination, (e) histopathologic evidence of mycobacterial infection including the presence of acid fast bacilli and/or granuloma at two or more anatomic sites other than the site of vaccination, and (f) having inconclusive results for the work-ups of other diseases.

The pathologic reports of 59 samples were available in the patients’ medical files, including 24 bone marrow aspirations or biopsies, 14 liver biopsies, 13 distant lymph node aspirations or biopsies, three skin rash biopsies, two bone biopsies, two lung biopsies and one gastric washing. No positive mycobacterial culture was detected among the patients. The competency of the immune system was evaluated by different tests including nitroblue-tetrazolium test, T-cell and B-cell counts by flow cytometry (FACScalibur, BD. US), Human immunodeficiency virus (HIV) test by enzyme-linked immuno-sorbent assay (ELISA) and evaluation of serum immunoglobulins and CH50.

We recruited patients’ pathology samples from pathology specimen banks. Twenty-eight samples from 21 patients, including nine smears from bone marrow specimens and 19 Formalin-Fixed and Paraffin-Embedded (FFPE) tissue specimens (i.e., six lymph node, nine liver, one subcutaneous nodule, one lung, one rib, and one smear of intra-abdominal abscess) were available. We deparaffinized FFPE specimens, as previously described (6). Nucleic acid extraction was performed on one to five sections (5 μm) of these specimens. We used proteinase K (Fermentas Co., Lithuania) lysis and phenol-chloroform (Sina gene Co., Iran) precipitation, followed by ethanol (Merck Co., Germany) precipitation to isolate deoxyribonucleic acid (DNA) from the smears and tissues. For differentiating *M. bovis* BCG from the other members of the *M. tuberculosis* complex, we performed an in-house multiplex PCR method to confirm the identity of the isolates, as described by Magdalena et al. (7) and Yeboah-Manu et al. (8).

The following primers were used: spacer region-specific primers for *M. bovis* BCG, spacer region 33 specific (5′ACACCGACATGCGGCGG3′) and spacer region 34 specific (5′CGACGGTTGGCGGAGG3′); the insertion sequence IS6110, MTC-specific primers, TB11 (5′GGACACGCGGTAATGCG3′), TB850 (5′TAGGGTCGGT-GACAAGGCCAC3′); and *Mycobacterium* genus-specific (65-kDa antigen gene) primers, TB1 (5′ACACGGATGTG-GTGCAT3′) and TB12 (5′CTTGTCGACCCGATACCC3′). All strains of *M. bovis* BCG produced two bands of 172 and 99 bp. The PCR conditions were 95°C for three minutes; 30 cycles at 95°C for 20 seconds, 65°C for 30 seconds, and 72°C for 30 seconds; and 72°C for seven minutes. The products were analyzed by electrophoresis on a 2% (wt/vol) agarose gel (8). Negative controls contained the PCR mixture without a DNA template and positive controls contained mycobacterial DNA extracted from the BCG vaccine.

The patients were classified into two categories, definite and probable cases, according to the working definition of disseminated BCG disease described by Talbot et al. in 1997 (5). The definite disseminated BCG infections met all three criteria, including: (i) identification of *M. bovis* BCG substrain by PCR, (ii) evidence of infection (including a positive culture, PCR or histopathologic demonstration of acid-fast bacilli) at two or more anatomic sites beyond the site of vaccination, and (iii) the presence of a systemic syndrome compatible with mycobacterial infection (such as fever, weight loss, and anemia). The probable case was defined based on all the criteria for a definite case except a positive PCR for *M. bovis* BCG (9). In the present study, the PCR result for *M. bovis* BCG was either negative or not performed in probable cases. The study design, conduct and assessments were all in accordance with ethical considerations, established and approved by the Ethics Committee of
Shiraz University of Medical Sciences. Continuous variables were compared using the Student t test. Data were analyzed using SPSS, version 11.5 (SPSS, Chicago, IL). Differences of P < 0.05 were considered statistically significant.

4. Results

There were 34 consecutive patients, 21 definite (Table 1) and 13 probable (Table 2) cases, reported from 1990 to 2007. All patients were healthy at birth and vaccinated routinely with the Pasteur 1173 M. bovis BCG substrain at the insertion of the deltoid. The patients were one to 60 months old with a mean (± SD) of 10.2 (± 12.5). Twenty-one patients (61.8%) were six months of age or younger. Thirty-one (91.2%) patients were younger than two years. About 41% (14 of 34) of the patients were male.

**Table 1.** Summary of Data on Twenty-one Definite Cases of Disseminated Bacillus Calmette Guerin Disease Reported From 1990 to 2007 in Shiraz, Southern Iran

| Cases | Year of Diagnosis | Gender | Age, mo | Systemic Syndrome | Immune Defect | Site(s) with the Evidence of Dissemination | Antimicrobial Therapy | Outcome |
|-------|-------------------|--------|---------|-------------------|---------------|------------------------------------------|-----------------------|---------|
| 1     | 1995              | M      | 16      | F, HSM, parotitis, GLN | CMI | DLN | I, R, S | A |
| 2     | 1995              | F      | 5       | F, P, skin nodules | SCID | Gastric washing, BM | I, R | D |
| 3     | 2003              | F      | 4.5     | F, HSM, GLN | None | Liver | I, R | A |
| 4     | 2003              | F      | 8       | F, HSM, GLN, weight loss | None | DLN, Liver | I, R | A |
| 5     | 2004              | F      | 6       | F, HSM, osteomyelitis | SCID | Rib | I, R, E, S | D |
| 6     | 2004              | F      | 13      | F, HSM, GLN | CGD | DLN, Liver, BM | I, R | D |
| 7     | 2004              | F      | 3       | F, HSM, generalized edema | SCID | BM, Liver | I, R | D |
| 8     | 2004              | M      | 5       | F, HSM, GLN | None | BM | I, R, E | D |
| 9     | 2004              | F      | 3       | F, HSM | SCID | Liver, BM | I, R | D |
| 10    | 2005              | M      | 4.5     | F, HSM, weight loss | None | Liver | I, R, E, S | A |
| 11    | 2005              | F      | 28      | F, HSM, GLN, abdominal mass | CMI | Intra-abdominal abscess, DLN | I, R | A |
| 12    | 2005              | M      | 9       | F, P, HSM | None | Lung | I, R, E | A |
| 13    | 2005              | F      | 6       | Bowel obstruction, GLN | None | DLN | I, R, E | D |
| 14    | 2005              | F      | 2       | F, HSM, sepsis | None | Liver | I, R, E, O | A |
| 15    | 2006              | M      | 1       | F, HSM, sepsis, skin nodules, | SCID | Skin nodule | I, R | D |
| 16    | 2006              | F      | 3       | F, P, HSM | SCID | Liver, BM | I, R, E, O | D |
| 17    | 2006              | F      | 17      | Weight loss, splenomegaly, Osteomyelitis | None | Bone, BM | I, R, E, O | A |
| 18    | 2006              | F      | 1.5     | F, GLN, Bowel obstruction | None | DLN, BM, Liver | I, R, E, O | D |
| 19    | 2007              | M      | 2       | F, P, HSM | None | BM | I, R, E, O, S | D |
| 20    | 2007              | F      | 4.5     | F, HSM | SCID | BM | I, R, E, O, S | D |
| 21    | 2007              | F      | 4       | F, sepsis, GLN | None | Liver, DLN | I, R, E, O, S | A |

Abbreviations: A indicates alive; BM, bone marrow; CGD, chronic granulomatous disease; C, clarithromycin; CMI, unidentified cell-mediated immune defect; D, died; DLN, distant lymphadenopathy; E, ethambutol; F, fever; GLN, generalized lymphadenopathy; HSM, hepatosplenomegaly; I, Isoniazid; IAD, lymphadenopathy; MO, months, O, ofloxacin; P, pneumonia; R, rifampin; SCID, severe combined immunodeficiency; S, streptomycin.

Evidence of dissemination included the histopathologic demonstration of acid-fast bacilli and/or typical histopathologic changes with granulomatous inflammation and/or the identification of Mycobacterium substrains, by standard polymerase chain reaction.
Fever in 31 (91.2%), axillary lymphadenopathy in 26 (76.5%) with fistulization in 50%, hepatosplenomegaly in 25 (73.5%), stunted growth in 21 (61.8%), distant lymphadenopathy in 16 (47.1%), cough (longer than three weeks) in 15 (44.1%), skin rash in 9 (26.5%) and vomiting in 9 (26.5%) were the most commonly reported signs and symptoms. Five patients (14.7%) had an active ulcer on their BCG injection sites. Disseminated BCG infection was diagnosed in three of the cases after laparotomy for bowel obstruction (cases 13 and 18, Table 1; case 5, Table 2). Specific pathologic findings (presence of granuloma and/or acid fast bacilli in tissue specimens) were found in 12 of 13 (92.3%) biopsies or aspirations of distant lymph nodes, 12 of 14 (85.7%) liver biopsy specimens and one of the two bone biopsy specimens. In the two lung biopsy specimens, pathologic diagnosis was based on the presence of granuloma. One gastric washing, done for a patient with pneumonia, and two of three biopsies of skin rash revealed acid fast bacilli (Tables 1 and 2). Specific pathologic changes with granulomatous inflammation and/or the identification of Mycobacterium substrains, by standard polymerase chain reaction.

The most common abnormal findings in blood profiles were as follows: anemia in 30 (88.2%), leukopenia (white blood counts less than 4000/μL) in 16 (47.1%), thrombocytopenia (platelet counts less than 150000/μL) in 15 (44.1%), and leukocytosis (leukocyte count that was two standard deviations above the mean count for a particular age) in 15 (44.1%). Of the 34 cases, 17 (50%) occurred in immunocompromised hosts. Severe combined immunodeficiency (SCID) was identified in 12 (35.3%), followed by unidentified cell mediated immune defect in 4 (11.8%) and chronic granulomatous disease in 1 (2.9%). HIV was not detected in any of the cases. Disseminated BCG infection developed in two brothers (case 8, Table 1; case 13, Table 2) and a brother and sister (cases 7 and 19, Table 1) among the patients.

Response to therapy was poor, with an overall mortality rate of 58.8% (20 of 34). Of the 20 patients who died of disseminated BCG infection, 15 (75%) had an immune defect. In contrast, of the fourteen patients who recovered, only two (14%) had an identified immunodeficiency.

Disseminated BCG infection was confirmed in 21 patients by PCR, which was directly applied to the 28 available clinical specimens in pathology specimen bank and one intra-abdominal abscess specimen (Tables 1 and 2) (10). PCR results were positive on 26 specimens for M. bovis BCG including 9 of 9 (100%) bone marrow smears slides, 16 of 19 (84.2%) of FFPE tissue specimens and one intra-abdominal abscess specimen. As for 16 FFPE tissue specimens, PCR results on 3 specimens from skin rash, bone and lung were positive for M. bovis BCG; and PCR positivity was 100% for distant lymph nodes (6 of 6) and 77.8% for liver (7 of 9).

5. Discussion

Herein, we report 34 patients with disseminated BCG infection, 21 definite and 13 probable cases and to the best of our knowledge, this is the largest study on...
children with definite disseminated BCG infection, reported from Iran. Disseminated BCG infection is a rare consequence of BCG vaccination, with a reported frequency of less than five per million vaccines and has mainly been seen in children with severe immune deficiencies (3). Adverse reactions to BCG vaccine appear to be grossly underreported (11). Approximately, 1.4 million children receive BCG vaccines every year in Iran and according to recent reports from Iran, the number of disseminated BCG infections is estimated to be higher than the expected rate (12-19). The greater reactivity of Pasteur 1173P2 strain vaccines, used in the national immunization program or higher prevalence of primary immunodeficiency may be possible explanations, which need further investigations (20, 21).

The majority of our patients (91.2%) were infants, consistent with previous reports in which 76-100% of the patients were under two years of age (5, 13, 17-19, 22). The most clinical presentations in our series were fever found in 31 (91.2%), axillary lymphadenopathy in 26 (76.5%) with fistulization in 50%, hepatosplenomegaly in 25 (73.5%), and stunted growth in 21 (61.8%), in agreement with previous reports (5, 13, 17-19, 22). Unusual presentation of disseminated BCG infection could be accompanied by gastrointestinal manifestations. Of the 34 patients, two cases presented bowel obstruction (cases 17 and 26, Table 1) and one with intussusception (case 5, Table 2). One patient with retroperitoneal abscess presented an abdominal mass, as reported previously (case 1, Table 1) (10). The histopathologic evidence of mycobacterial infection including the presence of acid fast bacilli and/or granuloma in tissue specimens removed by the liver and distant lymph node biopsy had very high yield of diagnosis (85.7% and 92.3%, respectively), so in children with suspected disseminated BCG infection in the absence of distant lymphadenopathy, liver biopsy could be suggested for early diagnosis. Besides, biopsy or smear of other organs such as the skin nodules, bone and lungs may serve as diagnostic tools, as previously reported (5, 23, 24).

The identification of M. bovis BCG substrain in tissue specimens of patients with suspected disseminated BCG infection is required for definite diagnosis. In this study, disseminated BCG infection was confirmed in 21 patients by PCR, which was directly applied to the 28 available clinical specimens with high rate of positivity (92.8%, 26 out of 28) (Tables 1 and 2) (10). In fact, the spread of BCG is a normal sequela of BCG vaccination, yet the identification and speciation of M. bovis BCG substrain by standard PCR in a patient with clinical findings consistent with disseminated BCG infection, could serve as a well-established criteria for the diagnosis of disseminated BCG infection (5, 9). Clinical management of BCG disease is difficult in the absence of standard clinical trials. As for our patients, response to therapy was poor, with an overall mortality rate of 58.8%, which differ significantly in immunocompromised cases and patients without an identifiable immunodeficiency (75% vs. 14%, respectively; \(P = 0.0003\)). The overall mortality rate of 71% was reported previously, despite aggressive management, and it ranged between 0% in patients without an identifiable immunodeficiency to 83% in immunocompromised counterparts (5).

Chemotherapy seems to be complicated by the inherent resistance of all M. bovis strains to pyrazinamide, the inherent intermediate resistance of some BCG strains to isoniazid, and the emergence of additional resistance during inappropriate therapy (25, 26). In a review in 1993, most M. bovis isolates from 73 patients were sensitive to isoniazid and rifampin, so during 1991 - 2004, the majority of our patients were treated with a combination of isoniazid and rifampin. Concern for the selection of antibiotic-resistance, led to a change from the two-drug combined therapy to three or more drugs since about 2005 in our cases (27). It has been suggested that four or more anti-tuberculosis drugs should be used for longer than one year in children with disseminated BCG disease until full recovery. The mortality in our patients who received different regimens of anti-tuberculosis was not significantly different (Tables 1 and 2). It seems that the patient's outcome is determined by their immunity state and/or progression of infectious process rather than anti-tuberculosis regimens. Therefore, investigation for a standard treatment of disseminated BCG infection seems mandatory. Several reports suggest that early bone marrow transplantation together with appropriate antimicrobials in immunocompromised patients is effective in restoring immunity and curing the infection (27, 28).

In the present study, about half of the patients were with identified with immunodeficiency. Despite a few cases of disseminated BCG infection reported in normal hosts, immunodeficiency has been detected in the majority of patients i.e., 86% in the review of Talbot et al. (5, 29, 30). Although, in our study, the majority of immunologic defects predisposed patients to mycobacterial infections, in our study, the majority of immunologic deficiencies except the defects of inrreleukin-12-interferon-\(\gamma\) (IL-12-INF-\(\gamma\)) axis predisposed patients to mycobacterial infections were investigated. The major defects of IL-12-INF-\(\gamma\) axis leading to Mendelian Susceptibility to Mycobacterial Diseases (MSMD) includes IFN \(\gamma\) R1, IL-12R \(\beta\) 1/IL-12 p40, and signal transducers and activator of transcription (STAT) 1 deficiency (31, 32). The major defects of the IL-12-INF-\(\gamma\) axis include IFN \(\gamma\) R1, IL-12R \(\beta\) 1/IL-12 p40, and signal transducers and activator of transcription (STAT) 1 deficiency (31, 32). Four patients with sibling relationship in our series were immunodeficient and all except one died despite aggressive treatment (cases no 7 and 8, Table 1 ; no 4 and 13, Table 2). Since most of the cases with disseminated BCG infection had immunodeficiency, mostly inherited as autosomal recessive, we suggest that all the siblings of cases with disseminated BCG infection should not be vaccinated at birth until complete evaluation and immunological work ups are performed.

In conclusion, infants and young children with prolonged fever, hepatosplenomegaly, lymphadenopathy
and a history of BCG vaccination should be examined for disseminated BCG infection. Polymerase Chain Reaction specific for M. bovis BCG in tissue specimens has a high rate of positivity in such cases. Nevertheless, further studies and clinical trials are required to assess the most appropriate anti-tuberculosis regimen (33).

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Footnotes

Authors’ Contribution: Abdolvahab Alborzi and Mohammad Hasan Aelami developed the original idea and protocol, abstracted and analyzed the data, and wrote the manuscript. Gholamreza Pouladfar developed the protocol, abstracted the data, and prepared the manuscript, and was the guarantor. Bita Geramizadeh, Bahman Pourabbas, and Jalal Mardaneh developed the protocol, performed the laboratory tests, and prepared the manuscript.

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