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Searching For Calmette - Guérin Bacilli in Bone Marrow Specimens of Vaccinated Children: A Real-Time PCR

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ABSTRACT

The Bacillus Calmette - Guérin (BCG) vaccine is purported to be disseminated in the body after inoculation via a lymphohematogenous route, even in immunocompetent individuals. This study was aimed to investigate the presence of Bacillus Calmette - Guérin in the bone marrow specimens of BCG vaccinated children using a molecular method. In this cross-sectional study, a total of 70 bone marrow samples were collected randomly from the hospitalized patients in Namazi and Amir Hospitals of Shiraz, Iran between April 2013 and March 2014. Bone marrow specimens were examined using the AmpliSens MD-FRT PCR kit (InterLabService Ltd, Moscow, Russia), designed for qualitative detection and differentiation of mycobacterium tuberculosis complex (M. tuberculosis, M.bovis, M.bovis BCG, etc.). Of the 70 cases, 42 (60%) were male. Patients' age ranged from 8 months to 6 years, with the most frequent age group being the 2-3 years (22.8%). Bone marrow aspirate smears showed no histopathological sign of granuloma/caseous granuloma. The bone marrow aspirate smears were also negative for acid-fast bacilli staining (i.e. Ziehl-Neelsen stain). Samples were all found to be negative for BCG based on real-time PCR results. Our study showed no evidence of bone-marrow involvement of BCG in a group of sick vaccinated children with no clinical presentation of disseminated BCG disease.

Key words: Calmette - Guérin Bacilli, BCG Vaccine, Bone Marrow, Real-time PCR

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INTRODUCTION

Bacillus Calmette - Guérin (BCG) vaccine is the only currently-available licensed vaccine against tuberculosis (TB) [1]. Since its discovery and initial testing in the 1920s, It's one of the most widely used live vaccines worldwide [2]. It is a live attenuated derivative of a virulent strain of Mycobacterium bovis and was originally derived from cows with tuberculous mastitis [1, 2]. BCG vaccination is a part of the immunization program in many developed and developing countries and is usually given early after birth or during the first 2 weeks of life [3]. It's been shown to provide up to 80% protection against severe tuberculosis such as miliary TB disease and TB meningitis [4]. Although BCG vaccination is generally considered safe, complications including subcutaneous abscess, osteomyelitis, regional lymphadenitis, eczema vaccinatum, hypertrophic scars, and keloid formation are reported to occur in <2% of vaccinated cases [5, 6]. The disseminated BCG infection is reported to have an incidence rate of 0.01-3.4 per million [7-9]. It is expected to occur mostly in immunocompromised children [10, 11]. After inoculation, BCG mycobacteria multiply both at the injection site, where they generate the induction of a prolonged inflammatory responses, as well as in remote sites with preponderance of local draining lymph nodes [12]. They are able to spread lymphohematogenously to distant parts of the body especially to reticuloendothelial system and bone marrow. In the state of immunodeficiency, this small confined foci of BCG in tissues escape from the detection/suppression

by the immune system and produce a clinical scenario known as systemic or disseminated BCG disease. The diagnosis can be confirmed by the isolation of M. bovis BCG through conventional and molecular methods from one or more clinical samples including regional lymph nodes, respiratory secretions, blood, cerebrospinal fluid, and bone marrow, in the presence of a clinical syndrome consistent with disseminated BCG disease [13]. If BCG can potentially disseminate to other body organs, could it be found in the bone marrow of vaccinated children? To answer this question, we have tested the bone marrow of BCG vaccinated children for the BCG bacilli. Since it wouldn't be ethical to ask healthy children to undergo an invasive and painful procedure such as bone marrow examination, we decided to include only the hospitalized pediatric patients who have undergone a bone marrow examination for some other clinical reason.

MATERIALS AND METHODS

In this cross-sectional study, a total of 70 bone marrow samples were collected randomly from the hospitalized patients in Namazi and Amir Hospitals of Shiraz, Iran between April 2013 and March 2014. Patients with an age < 6 years who had undergone a bone marrow examination for the clinical suspicion of hematologic disorders, malignancies and infectious diseases such as Kala azar, osteomyelitis, etc. in the period of time between April 2013 and March 2014 were included in this study. Any patients known or suspected to have a disseminated BCG disease were excluded.

This study was approved by the institutional review board of the Shiraz University of Medical Sciences and was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Chart review was done by skilled nurses and laboratory staffs were blinded to the patient characteristics and diagnosis.

Two- milliliters of bone marrow samples were collected in EDTA-coated tubes from all the participants, aliquoted and frozen at −80°C for real-time PCR assay. Considering that the Polymerase Chain Reaction (PCR) is a rapid, sensitive and specific method for detecting the BCG-type M.bovis, it was chosen to detect BCG genome in the bone marrow specimens. DNA extraction was performed on 250µl bone marrow samples. To achieve maximum yield, digestion was performed on one volume sample in two volumes 0.5% Tween 20, 0.5% NP-40, 10 mM NaOH, 10 mM Tris (pH 7.2), and 320 mg proteinase K per 1 mL (proteinase K lysis buffer) for 24 hours at 56°C and then boiled for 10 minutes followed by ethanol precipitation and resuspension in 100 µL sterile distilled water and storage at −80°C. Bone marrow specimens were examined using the AmpliSens MD-FRT PCR kit (InterLabService Ltd, Moscow, Russia), designed for qualitative detection and differentiation of mycobacterium tuberculosis complex (M. tuberculosis, M.bovis, M.bovis BCG, etc), according to manufacturer's instruction.

RESULTS

Of the 70 cases, 42 (60%) were male and 28 (40%) were female. Patients' age ranged from 8 months to 6 years, with majority being from the age group 2-3 years (22.8%) (Table 1).

Table 1: Patient characteristics

	Frequency (%)
Age	
<1 years	7 (10.0%)
1-2 years	15 (21.4%)
2-3 years	16 (22.8%)
3-4 years	14 (20.0%)
4-5 years	8 (11.4%)
5-6 years	10 (14.2%)
Male Sex	42 (60.0%)
Causes of BME	
FUO	10 (14.2%)
Kala azar disease	9 (12.8%)
Osteomyelitis	3 (4.2%)
Other infections	8 (11.4%)
Lymphoma (NHL, AML, ALL)	19 (27.1%)
Neuroblastoma	5 (7.1%)
Other malignancies	8 (11.4%)
ITP	4 (5.7%)

BME: bone marrow examination; FUO: fever of unknown origin; NHL: non-Hodgkin lymphoma; AML: Acute myeloblastic leukemia; ALL: Acute lymphoblastic leukemia; ITP: Immune thrombocytopenic purpura

Thirty (42.8%) patients were recruited from the Infectious diseases and Immunology wards of Namazi hospital and 40 (57.2%) cases were recruited from the hematology and oncology wards of Amir Hospital in Shiraz, Iran.

None of the patients' bone marrow aspirate smears showed caseous granuloma. The bone marrow aspirate smears were also negative for acid-fast bacilli staining (i.e. Ziehl-Neelsen stain).

Samples were all found to be negative for BCG based on real-time PCR results.

DISCUSSION

BCG vaccination is being implemented since 1921 in most regions of the world to immunize infants against TB. One hundred twenty million doses of BCG vaccine are being used each year around the globe [14-16]. Although not well investigated, BCG after inoculation is expected to undergo a course similar to mycobacterium tuberculosis after entry into lungs. Following multiplication and access to regional lymph nodes, mycobacterium BCG will probably have a lympho-hematogenous dissemination to other body organs. It is still unclear how this nonfatal dissemination of BCG after vaccination of infants can occur in the presence of an apparently intact immune system [17-19].

BCG dissemination in the immunodeficiency state is well-documented, however, there are also studies reporting it in apparently healthy individuals [20]. Likewise, there are several reports of BCG dissemination with bone marrow involvement in those with impaired immune system [21, 22]. In one case, a 68-year-old man had developed bone marrow infection with BCG two years after intravesical instillation of BCG for treatment of superficial bladder cancer. The bone marrow biopsy revealed granulomatous inflammation [21]. Many others have reported similar cases of disseminated BCG disease post intravesical treatment with BCG due to carcinoma in situ in the urinary bladder [23, 24].

There are more cases of BCG dissemination in apparently immunocompetent individuals in the literature [17, 19, 25-30]. The hypothesis is that after inoculation, BCG bacilli are phagocytosed. The macrophages that engulfed the bacilli can circulate within the body and seed them in reticuloendothelial tissues such as liver, spleen or bone marrow. Dissemination of BCG to not reticuloendothelial organs is also reported [31].

In 1956, Gormsen for the first time, proposed the hypothesis of hematogenous dissemination of BCG as a natural consequence of vaccination in humans [18]. He examined autopsy samples of 26 individuals and found granulomas in 13 of the samples in various organs, including the liver, lungs, spleen and kidneys but not in bone marrow [18].

In a study by Trevenen et al. at Children's Hospital, Winnipeg, autopsies from 36 infants (34 Canadian Indian, one Caucasian and one Inuit) that had been vaccinated with BCG shortly after birth were examined [19]. According to the results, 72.2% of infants had tuberculoid granulomas in many locations, including the vaccination site, regional lymph nodes, liver, spleen, lung, bone marrow and salivary gland. Only 2 (5.5%) had a tuberculoid granuloma formation in their bone marrow.

Our findings didn't show any evidence of the presence of BCG in the bone marrow of vaccinated children. We have used real-time PCR method, which is a highly sensitive and specific modality for detecting BCG-type M.bovis. One explanation could be the fact that small foci of BCG are sparse in the marrow and taking a tiny sample may not necessarily reach to it. So taking more specimens from each patient in a larger volume may have had a different yield. Studies which reported disseminated BCG disease with negative acid-fast bacilli or PCR findings could support that hypothesis [19]. The study population (i.e. pediatric patients who have undergone bone marrow examination for the suspicion of either a hematologic-oncologic or an infectious disease) may not be representative of all vaccinated children, but considering the invasive and painful nature of the bone marrow examination, it's unethical to be done on healthy children. Inadequate power could also be another potential reason.

CONCLUSION

BCG was not found in the bone marrow specimen of the sick vaccinated children without clinical manifestation of disseminated BCG infection. Further studies with larger sample size are recommended.

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REFERENCES

1. Fine PE: Variation in protection by BCG: implications of and for heterologous immunity. Lancet (London, England) 1995, 346(8986):1339-1345.
2. Aldwell FE, Tucker IG, de Lisle GW, Buddle BM: Oral delivery of Mycobacterium bovis BCG in a lipid formulation induces resistance to pulmonary tuberculosis in mice. Infection and immunity 2003, 71(1):101-108.
3. Hesseling AC, Johnson LF, Jaspan H, Cotton MF, Whitelaw A, Schaaf HS, Fine PE, Eley BS, Marais BJ, Nuttall J et al: Disseminated bacille Calmette-Guerin disease in HIV-infected South African infants. Bulletin of the World Health Organization 2009, 87(7):505-511.
4. Hanekom WA: The immune response to BCG vaccination of newborns. Annals of the New York Academy of Sciences 2005, 1062:69-78.
5. al-Bhlal LA: Pathologic findings for bacille Calmette-Guerin infections in immunocompetent and immunocompromised patients. American journal of clinical pathology 2000, 113(5):703-708.
6. Grange JM: Complications of bacille Calmette-Guerin (BCG) vaccination and immunotherapy and their management. Communicable disease and public health 1998, 1(2):84-88.
7. Casanova JL, Blanche S, Emile JF, Jouanguy E, Lamhamedi S, Altare F, Stephan JL, Bernaudin F, Bordigoni P, Turck D et al: Idiopathic disseminated bacillus Calmette-Guerin infection: a French national retrospective study. Pediatrics 1996, 98(4 Pt 1):774-778.
8. Dietrich G, Viret JF, Hess J: Mycobacterium bovis BCG-based vaccines against tuberculosis: novel developments. Vaccine 2003, 21(7-8):667-670.
9. Manissero D, Lopalco PL, Levy-Bruhl D, Ciofi Degli Atti ML, Giesecke J: Assessing the impact of different BCG vaccination strategies on severe childhood TB in low-intermediate prevalence settings. Vaccine 2008, 26(18):2253-2259.
10. Afshar Paiman S, Siadati A, Mamishi S, Tabatabaie P, Khotaee G: Disseminated Mycobacterium bovis infection after BCG vaccination. Iranian journal of allergy, asthma, and immunology 2006, 5(3):133-137.
11. Talbot EA, Williams DL, Frothingham R: PCR identification of Mycobacterium bovis BCG. Journal of clinical microbiology 1997, 35(3):566-569.
12. Cunha SS, Rodrigues LC, Pedrosa V, Dourado IM, Barreto ML, Pereira SM: Neonatal BCG protection against leprosy: a study in Manaus, Brazilian Amazon. Leprosy review 2004, 75(4):357-366.
13. Talbot EA, Perkins MD, Silva SF, Frothingham R: Disseminated bacille Calmette-Guerin disease after vaccination: case report and review. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 1997, 24(6):1139-1146.
14. Alkan M, Shoenfeld Y: BCG. In: Encyclopedia of Immunology. Volume I., edn. Edited by Roitt IM, Delves PJ. London, UK: Academic Press; 1992.
15. Ritz N, Tebruegge M, Streeton J, Curtis N: Too much of a good thing: management of BCG vaccine overdose. Vaccine 2009, 27(41):5562-5564.
16. Shahmohammadi SAS, Mohammad Jafar%A Rezai, Mohammad Sadegh: BCG-osis after BCG vaccination in immunocompromised children: Case series and review. Journal of Pediatrics Review 2014, 2(1):62-74.
17. Freundlich E, Suprun H: Tuberculoid granulomata in the liver after BCG vaccination. Israel journal of medical sciences 1969, 5(1):108-113.
18. Gormsen H: On the occurrence of epitheloid cell granulomas in the organs of BCG-vaccinated human beings. Acta pathologica et microbiologica Scandinavica Supplement 1956, 39(Suppl 111):117-120.
19. Trevenen CL, Pagtakhan RD: Disseminated tuberculoid lesions in infants following BCG vaccination. Canadian Medical Association journal 1982, 127(6):502-504.
20. Dederke B, Riecken EO, Weinke T: A case of BCG sepsis with bone marrow and liver involvement after intravesical BCG instillation. Infection 1998, 26(1):54-57.

21. Nemeth J, Stoiser B, Winkler HM, Mullauer L, Graninger W, Winkler S: Bone marrow infection with bacillus Calmette-Guerin (BCG) after intravesical immunotherapy. Wiener klinische Wochenschrift 2008, 120(3-4):121-123.
22. Xu Z, Roth VR: BCGitis induced inflammatory granuloma of the bone marrow. Blood 2014, 123(5):613.
23. Ersoy O, Aran R, Aydinli M, Yonem O, Harmanci O, Akdogan B, Pinar A, Sokmensuer C, Bayraktar Y: Granulomatous hepatitis after intravesical BCG treatment for bladder cancer. Indian journal of gastroenterology : official journal of the Indian Society of Gastroenterology 2006, 25(5):258-259.
24. Pommier JD, Ben Lasfar N, Van Grunderbeeck N, Burdet C, Laouenan C, Rioux C, Pierre-Audigier C, Meybeck A, Choudat L, Benchikh A et al: Complications following intravesical bacillus Calmette-Guerin treatment for bladder cancer: a case series of 22 patients. Infectious diseases (London, England) 2015, 47(10):725-731.
25. Katzir Z, Okon E, Ludmirski A, Sherman Y, Haas H: Generalized lymphadenitis following B.C.G. vaccination in an immunocompetent 12-year-old boy. European journal of pediatrics 1984, 141(3):165-167.
26. Kroger L, Brander E, Korppi M, Wasz-Hockert O, Backman A, Kroger H, Launiala K, Katila ML: Osteitis after newborn vaccination with three different Bacillus Calmette-Guerin vaccines: twenty-nine years of experience. The Pediatric infectious disease journal 1994, 13(2):113-116.
27. Lachaux A, Descos B, Mertani A, Souillet G, Gilly J, Hermier M: Generalized BCG infection, with a favorable outcome, in a 3-month-old immunocompetent infant. Archives francaises de pediatrie 1986, 43(10):807-809.
28. Pedersen FK, Engbaek HC, Hertz H, Vergmann B: Fatal BCG infection in an immunocompetent girl. Acta paediatrica Scandinavica 1978, 67(4):519-523.
29. Rositto A, Molinaro L, Larralde M, Ranalletta M, Drut R: Disseminated cutaneous eruption after BCG vaccination. Pediatric dermatology 1996, 13(6):451-454.
30. Tajima Y, Takagi R, Nakajima T, Kominato Y: An infant with asymptomatic hepatic granuloma probably caused by bacillus Calmette-Guerin (BCG) vaccination found incidentally at autopsy: a case report. Cases journal 2008, 1(1):337.
31. Raos M, Dodig S, Klancir SB, Kovac K, Hegedus-Jungvirth M: BCG dissemination in a child with normal immunity. Lijecnicki vjesnik 2000, 122(7-8):180-182.