

## ORIGINAL ARTICLE

### Inheritable Disorders Of Interferon-Gamma Interleukin-12 Pathway: Diagnosis And Management

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#### Abstract:

**Background:** Disseminated BCG infection is a typical clinical presentation in patients with an inherited disorder of the Interleukin (IL)-12-Interferon (INF)-Gamma axis, as BCG is often the first pathogen to which patients are exposed. BCG sub strains are derived from *Mycobacterium bovis*. BCG vaccination is routinely carried out in most regions of the world, with up to 95% coverage of children in Iran.

**Materials and Methods:** Sixteen patients were found with disseminated BCG disease, with the age range between 1 month-14 years. Diagnosis was based on positive culture of *M. bovis* -BCG variant from blood, sputum, gastric washing, and abscess or biopsy sites. Results of laboratory tests, radiological and CT-scan findings from the site of involvement were assessed and classified.

**Results:** Out of 16 cases, 63% of the cases (10 patients) were males while 37% (6 patients) were females. Mean age of the patients was  $4.75 \pm 3.39$  years old. The most common site of peripheral lymph node involvement was axillary (78%). Lung and para aortic lymph adenopathy were the commonest site of central involvement. Six patients (38%) had defects in *IL-12-INF-Gamma* axis of which three of them had *IL-12RBeta1* deficiency (mutation) and two had *IL-12p40* deficiency and one had *INF-Gamma R2* deficiency.

**Conclusion:** Considering the results of this study, it should be emphasized that prior to BCG vaccination, in cases with the family history of BCG-osis, all siblings should be evaluated for immune system disorders.

**Keywords:** Disseminated BCG, IL-12, Interferon gamma defect, Bacille Calmette-Guerin, Children, Iran, BCGosis

**Introduction:** Interferon-Gamma is a critical cytokine produced by Non-killer (NK) and T-cells. The differentiation of T-helper cells into Interferon (INF)-Gamma-producing cells is regulated by several cytokines, but principally interleukin-12 (IL-12). IL-12 is produced by antigen-presenting cells (particularly dendritic cells and macrophages) in response to infection. IL-12 not only promotes T-helper cell differentiation, but also induces INF-Gamma production in other cells, such as NK cells. Deletions in germline mutations in five genes involved in the IL-12-INF-Gamma circuit have been found in human patients: IFNGR1, encoding the ligand-binding chain of the INF-Gamma receptor (INF-Gamma r1); IFNGR2, encoding the associated chain of the INF-Gamma receptor (INF-Gamma r2); STAT1, encoding the signal transducer and activator of transcription-1 (STAT1) in the INF receptor signaling pathway; IL12B, encoding the beta 1 subunit shared by the IL-12 and IL-23 receptors (IL-12RBeta1). (1) Disseminated BCG infection is a typical clinical presentation in patients with an inherited disorder of the IL-12-INF-Gamma axis, as BCG is often the first pathogen to which patients are exposed. BCG substrains are derived from *Mycobacterium bovis*. BCG vaccination is routinely carried out in most regions of the world, with up to 95% coverage of children in Iran. BCG prevents severe forms of child-

hood tuberculosis (TB), including miliary tuberculosis and meningitis in particular; however, in rare patients, BCG vaccination results in disseminated infection involving lymph nodes, lungs, kidney, spleen and other organs. Such infections are referred to as "BCG-osis" and are complications of BCG injection, with high (71%) rates of mortality (2).

BCG-osis invariably indicates the presence of an underlying congenital or acquired immune deficiency, such as severe combined immunodeficiency (SCID), chronic granulomatous disease (CGD) or HIV infection. Patients with these conditions are also vulnerable to various other microbes. Of the remaining patients with BCG-osis, half present MSMD (mendelian susceptibility to mycobacterial disease). About half of the known MSMD patients have been shown to present an inherited defect of the IL-12-INF-Gamma axis, whereas the remaining cases remain asymptomatic. (3)

We report here the cases of BCG-osis with hereditary defects in the IL-12-INF-Gamma axis and describe the clinical, paraclinical and molecular features.

**Materials and Methods:** In this study, clinical, laboratory, radiological and molecular data were obtained from the medical records of all patients with Disseminated BCG disease admitted in the pediatric ward at National Research Institute of Tuberculosis and Lung disease (NRITLD), a referral center for Tuberculosis and lung disease, located in Tehran capital of Iran, during a 8 year period (1999-2007). Medical history including personal and family history, physical examination data, growth and development chart, site of lymphadenopathy were obtained in all patients. Diagnosis was based on positive culture of *M. bovis*, BCG variant, from blood, sputum, gastric washing, and abscess or biopsy sites. Results of laboratory tests, radiological and CT-scan findings from the site of involvement were assessed and classified. Induration's of more than 10mm by tuberculin skin test (Mantoux test by intra dermal injection; 0.1 ml of 5 tuberculin units) was taken as positive. Analysis of peripheral blood B lymphocytes (CD19), T lymphocytes (CD3), T cell subpopulations (CD4, CD8), natural killer cells (CD56, CD16) and also of serum immunoglobulins was done.

The ability of interferon-Gamma to up-regulate the production of TNF-Alpha by monocytes was studied with an in vitro whole-blood assay. The production of TNF-Alpha in response to *Escherichia coli* lipopolysaccharide (1 microgram per milliliter) was compared with TNF-Alpha production induced by the same concentration of lipopolysaccharide after pretreatment with interferon-Gamma (2 microgram per milliliter) for two hours. Plasma TNF-Alpha levels were measured with an enzyme-linked immunosorbent assay.

**Results:** Sixteen patients were found with disseminated BCG disease, with the age range between 1 month-14 years and mean age of  $4.75 \pm 3.39$  years old. Male: Female ratio was 10:6. Clinical and radiological features are depicted in Table 1. Scar of previous BCG vaccination was observed on the right arm of all children. On an average the onset of symptoms appeared

at 5.5 months of age. Considering the growth chart, 50% (8 cases) were below the 10<sup>th</sup> percentile curve. Abscess at the injection site was reported in all of the cases. In CT-scan, abdominal involvement was observed in 10 patients (71.4%). In this group, 100% had paraaortic lymphadenopathy, with retroperitoneal lymphadenopathy in one case. 20% of patients had ascites, splenomegaly and abscess formation were seen in 2 cases. In one case hyper resonance nodules was present in the spleen and in another one hyperechoic mass was detected in liver. Tuberculin skin test was positive in 6(37.5%) children.

8 children (50%) demonstrated hypogammag-lobulinemia (IgG <1000 & IgA<100). Lymphocyte subsets were low in 31 % ( 5 cases). Direct smear of gastric washing for AFB were positive in 25 % ( 4 cases), culture of gastric lavage was positive in 12.5 % ( 2 cases), results of PCR sampling on gastric washing for *M.bovis* were positive in 18.7 % ( 3 cases). Direct smear and culture for AFB on lymph node sampling were positive in 18.7 % ( 3 cases). Pathologic findings compatible with chronic mycobacterial granulomatous tissue with necrosis were seen in 63% (10 cases) of lymph node biopsies.

Six patients (38%) had defects in *IL-12-INF-Gamma* axis of which three of them had *IL-12RBeta1* deficiency (mutation) and two had *IL-12p40* deficiency and one had *INF-Gamma R2* deficiency. All patients had negative serology for HIV infection.

All patients received the anti TB medication (Isoniazid, Rifampicin and Ethambutol). Meanwhile in five cases due to poor clinical response and progression of the disease, in addition to the above anti TB regimens, Clarithromycin and Ofloxacin were added. Gamma interferon was used in one case. All the patients are alive and are under regular follow-up.

**Table 1: Clinical and radiological features of children with BCGosis**

Lymphadenopathy	100%
Right axillary adenopathy	31%
Hilar adenopathy	25%
Mediastinal adenopathy	19%
Left axillary adenopathy	13%
Fever	85%
Weight Loss	80%
Splenomegaly	53%
Pneumonia	42%
Pulmonary abscess	7%
Skin involvement	7%
Pulmonary Nodule	7%
Lobar consolidation	4%

**Discussion:** Immunization of children with Bacillus Calmette-Guerin (BCG), a live attenuated bacterial vaccine derived from Mycobacterium Bovis, is recommended by the World Health Organization in communities with a high prevalence of tuberculosis. (1,2) BCG vaccines are extremely safe in immunocompetent hosts, but possible complications range from local inflammatory reactions (lymphadenitis, abscess and fistula formation) to disseminated diseases (osteomyelitis, bacteremia, meningitis) and death.(3) We found disseminated BCG more common in boys as compared to a study by Deeks et al in Canada during 2005 where the incidence of disseminated BCG disease was slightly higher in girls as compared to boys (4).

In Iran, BCG vaccination is performed immediately after birth. It has been shown in different studies that the complications of BCG vaccination appear within 6 months of birth. (5, 6) In this study mean age for the appearance of the symptoms was 5.5 month. In one case, symptoms appeared at 6 years of age. Most of the clinical signs in our study comprised of fever, weight loss and lymphadenopathy. Also in study by Casanova et al, fever and cachexia were common in the patients. In our study 78.6% had pulmonary and 71.4% suffered from abdominal involvement. Casanova and his colleagues demonstrated lymph node involvement in 94%; with 69% having pulmonary involvement. (7)

Other studies also have demonstrated abdominal involvement in more than 30% and renal involvement in 19% of the cases. In our study, one of the patients (7.1%) had involvement of the urinary system.

BCG vaccine is contraindicated for people with immunodeficiency diseases. Most of children with disseminated disease have immunodeficiency disorders and should not receive the vaccine if their condition is known. However, the vaccine is given in the neonatal period when it is often difficult to elicit a history of or detect immunodeficiency disorders. This is particularly true for conditions such as SCID, CGD, IL-12-INF-Gamma axis, for which no neonatal screening test is routinely performed (8, 9,10)

The records of our review showed that most of patients with disseminated BCG disease had immunodeficiency. Immune defects in the IL-12-INF-Gamma axis were identified in six subjects, also genetic study for INF-Gamma receptor deficiency is in progress. In a study by Farhoudi, et al. one of the 6 cases had INF-Gamma receptor deficiency. (11) In the retrospective review of disseminated BCG disease by Lotte, et al, all the 60 patients took part in that study, had cellular immunodeficiency and 31 of these patients died. (12) Additionally, in study of Talbot et al immunodeficiency was identified in 24 out of 28 subjects.(1)]

Recently, patients with severe infections due to otherwise poorly pathogenic mycobacteria (non-tuberculous mycobacteria or Mycobacterium bovis BCG) or Salmonella species have been identified with IL-12 IFN Gamma receptor deficiency. Many of these patients were unable to produce or respond to interferon gamma. Unusual mycobacterial infections were also reported in several patients with genetic defects in inhibitor of NFkappaB kinase gamma, a key regulatory molecule in the nuclear factor kappaB pathway. (14) The first seven cases of IL-12 RBeta1 deficiency were published in 1998. Eight years later, 89 IL-12R Beta1

deficient patients have been described, including 62 published cases. IL-12RBeta1 deficiency is therefore the most frequent known genetic etiology of MSMD. Forty-one mutant alleles have been identified, 29 of which have been published. All mutant alleles are recessive, loss-of-function and cause recessive complete IL-12RBeta1 deficiency. In this study we report three patients with BCG-osis with *IL-12RBeta1* deficiency, mycobacterial disease and salmonellosis are the most frequent infectious disease in patients with IL-12RBeta1 deficiency. Like IL-12p40-deficient patients, about half of all the known IL-12RBeta1-deficient patients have developed Salmonella infection. Infectious disease occurred before the age of 12 years in symptomatic patients, as in patients with RC-INF-Gamma R1 or INF-Gamma R2 deficiency. However, unlike these patients, the clinical outcome was relatively good, with only 17% deaths, and most patients surviving into adulthood, although our three patients are in childhood period. (15,16)

All known IL-12B mutations are recessive and loss-of-function, resulting in recessive complete IL-12p40 deficiency with a lack of detectable IL-12p40 secretion by the patients' blood cells and EBV-transformed B cells. Our patient with IL-12p40 deficiency presents a novel mutation in IL12B, indicating that IL-12 deficiency is not restricted geographically and that the spectrum of mutations is not as limited as previously thought. The patient suffered chronic infection with a reasonably good outcome. We suggest that the overall prognosis of such cases is good, with broad resistance, low penetrance of the mutation and a favorable outcome regarding of infection.

*INF-Gamma R2* deficiency is one of the rarest genetic etiologies of MSMD: only nine children have been identified, including seven children from the six families reported to date. The first patient was reported in 1998. This child and six other patients had recessive complete (RC) INF-Gamma R2 deficiency. Two forms of RC INF-Gamma R2 deficiency were documented. The study of INF-Gamma R2 deficiency has had unexpected genetic implications, beyond the field of MSMD and even that of primary immunodeficiencies. (17, 18)

In one of our patients investigation of the IL-12-INF-Gamma axis by means of a recently developed whole-blood cells in response to BCG plus INF-Gamma sequencing of the *INFGR1*, *INFGR2*, and *STAT1* genes revealed that the patient was homozygous for a missense mutation in *INFGR2* (T168N). The parents were heterozygous for this mutation, which was not found in 100 healthy controls tested. The pathogenic effects of this mutation were shown to be due to the creation of a novel N-glycosylation site in INF-Gamma R2. The receptors were expressed on the cell surface, defining a novel form of INF-Gamma R2 deficiency. These data unambiguously demonstrated the presence of an autosomal recessive, complete INF-Gamma R2 deficiency in this patient. By analogy with other INF-Gamma R2-deficient patients and the larger number of patients with complete INF-Gamma R1 deficiency, this patient probably has a poor prognosis, despite his current clinical remission.

Inherited *INF-Gamma R1* deficiency was the first genetic etiology of MSMD to be identified, in 1996.(18) In the last 10 years, 30 different *INFGR1* mutations have

been identified in 86 patients world-wide. INF-Gamma R1 deficiency is a very severe condition, with an early onset of infection and a poor prognosis.(19) Children are mostly infected by BCG and environmental mycobacteria, notably rapidly growing mycobacteria such in our patient with INF-Gamma R1 deficiency the type of bacilli was mycobacterium rapid growing.(20) The clinical penetrance of INF-Gamma R1 deficiency is complete in childhood, and the mean age at onset of first infection is 3.1 years compared to our patient the onset of symptoms was 2 year old. Most of the affected children died in childhood and only four of the 22 published patients reached the age of 12 years, our case now is 6 years old. Antibiotic treatment does not give full and sustained clinical remission and INF-Gamma has no effect in the absence of a functional receptor. (21)

In various studies, the rate of mortality and morbidity among children with disseminated BCG disease is greater than 70%. (22) Also in Casanova, et al. study the prognosis of BCG infection was poor and mortality rate reported as 43%. In the study of Afshar Paiman, et al. most of their patients died despite aggressive management. (1) However in our study, one of our patients expired due to dissemination of BCG infection.

Treatment of these diseases include: anti bacterial, anti tuberculosis in addition to INF-Gamma in selected cases. More advanced treatment procedures, such as bone marrow transplantation or gene therapy, might improve the prognosis of such patients in the future. (20)

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