http:// ijp.mums.ac.ir
Original Article (Pages: 10595-10603)

The Relationship of Serum Levels of Gamma Interferon and its Receptor (CD119) to Development of BCG Axillary Lymphadenopathy

Mohammad Ali Zamani[1], Hajar Sorani[2], *Ali Asghar Rabiei[3], Mehdi Ghatreh-Samani[4], Soleiman Kheiri[5], Nika Khoshdel[6], Abolfazl Khoshdel[7]

[1]Assistant Professor, Department of Pediatrics, Shahrekord University of Medical Sciences, Shahrekord, Iran. [2]Department of Pediatrics, Shahrekord University of Medical Sciences, Shahrekord, Iran. [3]Assistant Professor, Department of Pediatrics, Shahrekord University of Medical Sciences, Shahrekord, Iran. [4]Department of Microbiology and Immunology, Shahrekord University of Medical Sciences, Shahrekord, Iran. [5]Professor, Department of Biostatistics and Epidemiology, Faculty of Health, Shahrekord University of Medical Sciences, Shahrekord, Iran. [6]Medical Student, Shiraz University of Medical Sciences, Shiraz, Iran. [7]Professor, Department of Pediatrics, Clinical Search Department Unit, Hajar Hospital, Shahrekord University of Medical Sciences, Shahrekord, Iran.

Abstract

Background: Lymphadenitis is the most common complication of BCG vaccination in children. Interferon-gamma (IFN-γ) plays a key role in immune response to Mycobacterial infections. In this study, the relationship of serum levels of IFN-γ and its receptor (CD119) to development of Bacillus Calmette Guerin (BCG) axillary lymphadenopathy was investigated.

Materials and Methods: In this case-control study, 45 children with axillary lymphadenopathy and 45 healthy children matched by age and sex were included. Two ml peripheral blood was collected in tubes containing anticoagulants. Then, level of IFN-γ was measured by ELISA and the level of CD119 expression in the peripheral blood mononuclear cell (PBMC) was measured by flow cytometry. Data were analyzed using SPSS software version 22.0.

Results: Totally, 90 children were enrolled in this study, which consisted of 30 girls and 60 boys. The mean age of participants was 14.5±6.5 months in case group and 15.2±7.1 months in control group, respectively (p=0.61). The level of IFN-γ was significantly lower in case group than in control group (p<0.001), but no significant difference was observed in PBMC percentage between the two groups (p>0.05). There was no significant relationship of age and sex to BCG (INF and PBMC) lymphadenopathy (p>0.05).

Conclusion: Based on the results, IFN-γ level was significantly lower in the BCG lymphadenopathy group than in the control group. Levels of IFN-γR (CD119 cellular level in PBMC) in two groups did not show a significant relationship.

Key Words: Bacillus Calmette Guerin, CD119, Children, Interferon gamma, Lymphadenopathy.

*Please cite this article as: Zamani MA, Sorani H, Rabiei AA, Ghatreh-Samani M, Kheiri S, Khoshdel N, et al. The Relationship of Serum Levels of Gamma Interferon and its Receptor (CD119) to Development of BCG Axillary Lymphadenopathy. Int J Pediatr 2019; 7(12): 10595-603. DOI: **10.22038/ijp.2019.44335.3671**

Corresponding Author:

Ali Asghar Rabiei (M.D), Department of Pediatrics, Shahrekord University of Medical Sciences, Shahrekord, Iran.

Email: aarabiei@gmail.com

Received date: Aug.12, 2019; Accepted date: Nov.22, 2019

1- INTRODUCTION

Tuberculosis (TB) due to *Mycobacterium tuberculosis* (Mtb) is currently one of the main health issues across the globe (1, 2). Data from 202 countries (the Global Tuberculosis Report 2014) indicated the presence of TB in all regions of the world (3). Most patients with Mtb infection exhibit no clinical disease symptoms and less than 10% of them have history of developing clinical TB (4). Bacillus Calmette Guerin (BCG) vaccine is a live attenuated vaccine (strain of *Mycobacterium bovis*) that has been administered worldwide to prevent TB (5).

BCG was developed by Albert Calmette and Camille Guerin in France between 1908 and 1921. According to World Health Organization (WHO) recommendation, BCG vaccination should be accomplished for all infants in several countries, especially in highly endemic countries or countries neighboring such regions (6). The efficacy of BCG vaccine to prevent TB is indefinite; this vaccine protects against the meningeal/miliary TB in childhood TB but not against adult TB (5). Although BCG vaccination is safe for most children (7), it may lead to certain complications such as abscesses and cellulitis at the site of inoculation and regional lymphadenitis (8). The most widespread complication of BCG vaccination is lymphadenitis (5).

BCG vaccine induced lymphadenitis refers to the enlargement of ipsilateral regional lymph node (particularly the axillary lymph nodes and rarely neck lymph nodes) following BCG vaccination so that it can be touched, without local/systemic signs of inflammation (2). In the normal course of the disease, BCG lymphadenopathy can be self-limiting within a few weeks or progress and develop into purulent lymphadenitis (5). The prevalence of lymphadenopathy, purpura lymphadenitis and fistulatization in certain regions of Iran has been reported to be 2.2%, 0.5% and 1.8%, respectively (9). The immune response to BCG lymphadenitis has not yet been exactly explained. Similar to the primary infection, if a person vaccinated with BCG is contaminated with Mtb, a severe cellular immune response will occur and the infection will be controlled. Therefore, the aim of immunization with BCG is not to prevent Mtb infection, but to localize the infection and prevent its spread (10). Generally, the cellular response to mycobacterial infection is exhibited through IFN-γ produced by T cells, which activates macrophages to kill Mtb (11).

IFN-γ, which is the most important cytokine, is from type II family. IFN-γ is mainly produced by Th1 (CD4 and CD8), natural killer (NK) cells and natural killer T (NKT) cells (12). Recently, IFN-γ release assays (IGRAs) have been developed to detect latent tuberculosis infection. This series of experiments measures individual immune response by measuring the release rate of IFN-γ from T cells after stimulation by Mtb-specific antigens (ESAT-6 and CFP-10).

These antigens are absent in BCG and most peripheral mycobacteria (13). The use of IGRA in children is controversial yet remains widely accepted (14). Interferon Gamma Receptor-1 (IFN-γR1), also called CD119, is a protein in humans transcribed by the IFNGR1 gene (15). It has been reported that mice with IFN-γ- or the IFN-γR-deficiency are highly predisposed to infection with tuberculosis-causing organisms (16); but the effect of IFN-γ in human macrophages remains controversial (11). Although many clinical studies have been conducted on IFN production in TB, studies of IFN-responsiveness are rare. The functional and genetic defects of the IFN-γR have not been identified in BCG lymphadenitis and no convincing clear evidence is available to approve that IFNγ contributes greatly to most clinical TB infections (17).

Therefore, the aim of this study was to investigate the relationship of IFN-γ levels and the cellular expression levels of serum CD119 to the development of BCG axillary lymphadenopathy in patients and controls. The results of this study are expected to help clinicians to predict lymphadenitis, prevent indiscriminate treatments and prescription of unnecessary antibiotics, and adopt specific treatments.

2- MATERIALS AND METHODS

2-1. Study design and population

This is a case-control analytical study conducted from April 2017 to March 2019. Samples were selected by available sampling from patients referring to Imam Ali Clinic, Shahrekord, Iran, during the period 2016 to 2019. Per each patient, a healthy child was included as a control among patients who referred to Imam Ali clinic in Shahrekord who were included in the study. According to previous studies, 45 children with axillary lymphadenopathy and 45 healthy children were enrolled (18, 19). The control group was matched by age and sex with the case group.

2-2. Laboratory measurements

Blood samples were collected from all samples and serum IFN-γ was measured by ELISA. The cellular expression of the CD119 was measured using specific antibody with flow cytometry device.

2-3. Intervention

2-3-1. Vaccination methods

The dry BCG vaccine was injected at an interval of one-third upper and lower thirds of the arm at 0.05 dosage and for the two groups methods of vaccination were the same.

2-3-2. The steps of measuring serum IFN-γ levels by enzyme linked immunosorbent assay (ELISA)

Blood samples (3 ml) were centrifuged for 1000-1200 minutes to conduct an ELISA test in clotted gelated tubes. Serum was stored at -70 ℃ in microtubes until conduction of ELISA. Hemolytic or lipemic sera or serum samples with rare diseases were excluded and replaced with other appropriate samples. Positive control was considered to be PHA (phytohaemagglutinin, 1 mA), negative control to be antigen-free and the test to contain specific bacillus antigens was considered (20). The experiment steps were followed according to the instructions of an Elisa kit (R & D, Minneapolis, MN, USA), and continued via preparation of the standard solution, IFN-γ antibody and streptavidin-HRP. An incubation period at 37 °C was done and the wells were washed with buffer four times, each time for 30 s. After adding chromogenes A and B to all wells, 10 min of incubation, and finally adding of a stopper, optical absorbance was read at 450 nm wavelength using stat fax 2100 ELISA reader (7).

2-3-3. Flow cytometry analysis to determine IFNγ-R1 (CD119) marker on PBMC

The separation of peripheral blood mononuclear cells (PBMCs) was performed on healthy and patient participants using the Ficoll method and concentration gradient. Two ml of peripheral blood containing anticoagulant was diluted in an equal amount of PBS (phosphate buffered saline), and slowly poured into two ml of Ficoll solution (Inno-train, Germany). After centrifugation, the lymphocyte zone was inserted between the plasma and the Ficoll layers based on the density gradient. This layer was was isolated carefully. The isolated monoclonal cells were washed with PBS solution in three steps to remove all of the extra substances and proteins. To determine the frequency of the CD119 marker, specific anti-monoclonal antibodies against CD119 cellular level were used. To all the tubes, about 1 million

PBMC cells were added and then 3 Landa of the respective antibody was added to the test tubes. The negative control tube contained blood without labeled antibodies. All tubes were incubated for 20 min at room temperature. Then 1000 µl of non-sterile PBS was added to each tube. After centrifugation, a solution was obtained containing approximately 1,000,000 cells stained with fluorochrome antibodies. The tubes were promptly read by a flow cytometry device (Cyflow PARTEC). Anti-human CD119-PE (eBioscience Company) was used. CD119 was reported as PBMC percentage.

2-4. Ethical consideration

This research project was approved by the Ethics Committee of Research and Technology Department, affiliated with Shahrekord University of Medical Sciences with ethical number: IR.SKUMS.REC.1396.184 and written consent to participate in the study was obtained from parents.

2-5. Inclusion and exclusion criteria

Case group was 45 children aged 7-36 months that developed axillary lymphadenopathy on the site of injection following BCG vaccination. The inclusion criteria were children with axillary lymphadenopathy (enlargement of the armpit lymph nodes without fever or other clinical symptoms), and a history of BCG vaccination (14). Patients were excluded from the study if they had immunocompromised disorders and family (sibling) history of immunodeficiency (BCGitis and history of immunodeficiency) or withdrew from participation in the study.

2-6. Data Analyses

All data were recorded in the checklist and entered into the SPSS software version 22.0. Quantitative variables with normal distribution were expressed as mean ± standard deviation (SD), and quantitative variables with non-normal distribution as median and interquartile range. Comparison of age between the two groups was done by independent t-test and comparison of IFN-γ level and CD119 expression levels between the two groups was carried out by the Mann-Whitney test. P-value less than 0.05 were statistically significant.

3- RESULTS

In this study, the following results were obtained regarding the relationship of IFN-γ levels and serum CD119 expression to the occurrence of BCG induced axillary lymphadenopathy in Shahrekord, Iran. A total of 90 children aged 7-36 months with an average age of 14.9 ± 6.8 months were included in this study, of whom 45 were assigned to case group (children with axillary lymphadenopathy), and 45 to control group (healthy children). Each group consisted of 15 girls and 30 boys.

The mean age was 14.5 ± 6.5 months in the case group and 15.2 ± 7.1 months in the control group, without any statistically significant difference (P=0.61). The results of the comparison of the studied variables including IFN-γ and PBMC percentage are shown in **Table.1**. As seen, the mean IFN of the case group was significantly lower than that of the control group (P <0.001). There was no significant difference in PBMC percentage between the case and control groups (P>0.05). Furthermore INF and PBMC percentage were not significantly associated with age and gender in the current study (P<0.05).

Table-1: Results of vaccine response parameters in case and control groups (n=90).

Variables	Case group (n=45)	Control group (n=45)	P-value
	Median (interquartile range)	Median (interquartile range)	
Interferon gamma concentration (ng/ml)	22.6 (15.7-66.25)	163.4 (68.7-294.5)	<0.001
PBMC	62.55 (47.15-69.25)	62.3 (53.4-68.8)	0.884

PBMC: peripheral blood mononuclear cells. Case group: children with axillary lymphadenopathy, Control group: healthy children.

4- DISCUSSION

In this study, the relationship of serum levels of IFN-γ and its receptor (CD119) to develop BCG axillary lymphadenopathy was investigated. In the present study, the mean age of children with BCG lymphadenopathy was 9.9 ± 8.6 months. There was no significant relationship of age and sex to BCG (INF and PBMC) lymphadenopathy (P>0.05). Consistent with the present study, in Behjati and Ayatollahi's study (9), there was no significant difference in the incidence of lymphadenopathy between boys and girls. However, in one study the prevalence of disseminated BCG infection was reported to be higher in males; however, the sample size of that study was extremely low (21).

Various factors such as age at vaccination, vaccine injection site, vaccine preparation, immune system failure, and race, different strains of BCG, and manufacturer and individual characteristics of the population studied were factors influencing the incidence of complications of BCG vaccine (including lymphadenopathy and lymphadenitis). Incorrect technique of intradermal injection and excess dose increase the likelihood of developing complications (22-24). In the present study, IFN-γ was significantly lower in BCG lymphadenitis group than in control group. In the majority of studies, IFN-γ levels have been higher in patients with active TB compared to those with latent TB (25), and in some studies, an opposite finding has been obtained (26). Kim et al. reported that in patients with positive IGRA test, IFN-γ levels in those with TB were significantly higher than those without TB, and therefore suggested IGRA as a method for the diagnosis of TB (27). Following TB infection, despite phagocytosis by macrophages, the bacilli that have been introduced into the macrophages continue to proliferate in the early stages, and are induced only after the antigen is supplied to T cells (CD4 and CD8), IFN-γ and IL-2 (28). Macrophages are contaminated and monocytes are activated in the presence of IFN-γ which leads to intracellular bacterial death by increasing the phagosome fusion with lysosomes (13). The results of this study indicating the lower level of IFN-γ in children with BCG lymphadenitis than in those without lymphadenitis are consistent with previous studies. However, in the present study, the effects of BCG vaccine but not active TB have been investigated.

In some studies, production of IFN-γ after BCG vaccine has not been directly related to resistance against the disease, but has served as an important predictor of the effectiveness of the vaccine and the severity of the disease (29). Similar to the present study, in a study in 74 TB cases in Iraq; the mean IFN-γ serum level significantly decreased (26.92±5.29 pg/ml) in comparison to healthy controls (28.40±10.73pg/ml). A decrease was also observed in IFN-γ level during TB infection (24), but in the TB patients aged 15-60 years, mean of IFN-γ was

48.69+28.78pg/ml while it was 12.99+5.70pg/ml in the control group. It was argued that measurement of IFN-γ production is helpful to diagnose active TB yet additional studies are still needed (13). Condos et al. reported that IFNγ signal transduction system is functionally normal in the alveolar macrophages of TB patients (30). Similar to the present study, in some studies a significant relationship has been noted between IL and IFN deficiency in children experiencing severe complications of BCG vaccination in northern Iran (19). IL-17 and IFN-γ levels were significantly lower while IL-4 level was higher in patients suffering from active TB (26). We should have considered the role of other cytokines in our study as well. Perhaps the deficiency of or changes in other factors and the immunity level of children in the region have confounded our findings. It should be noted that the diagnosis of TB is difficult in patients with BCG lymphadenitis.

Tuberculin skin test (TST) is not useful for the diagnosis of BCG lymphadenitis. TST is expected to be positive after recent BCG vaccination in a person with complete immunity. BCG lymphadenitis is characterized by positive TST and, if necessary, negative IGRAs test, accompanied by normal X-ray chest, no fever, and unilateral involvement of lymph nodes (mostly in the armpit). TB specimens isolated from the armpit lymph nodes are rare (9, 14).

Studies regarding the diagnostic accuracy of IGRA for TB lymphadenitis have reported contradictory results. The contradiction of previous studies' findings can be, to some extent, attributed to small sample size of separate studies indicating lack of statistical power. The diagnostic value of IGRA differed by different IGRA methods, clinical courses ethnicity and lymphadenitis location (31). It should be noted that the IGRA method is based on the response of T lymphocytes susceptible to specific Mtb antigens, i.e., ESAT-6 and CEP10 (Culture Filtrate Protein (11). These antigens are encoded on genes located in region of difference 1; Mtb. The gene is absent in *M. bovis*, BCG and most NTMs, and therefore has lower cross reactivity in BCG-vaccinated individuals and does not secrete IFN-γ (32). Consequently the probability of false positive results is extremely low in the IGRA test (25). IGRAs are more specific and are not affected by BCG vaccination (33). IFN-γ deficiency has been reported in patients with acquired immunodeficiency syndrome (AIDS), and patients with primary immunodeficiency disorder. Disseminated BCG infections have also been reported in patients with immunodeficiency (22, 34).

Therefore, the deficiency may be associated with systemic complications of disseminated BCG infections. In the present study, there was no significant difference in PBMC between the BCG lymphadenopathy and control groups ($P<0.05$). Farhondi et al. studied disseminated BCG infection in 40 patients. In that study, IFN-γR or CD119 was checked in six patients, one of whom was diagnosed with IFN-γR deficiency using flow cytometry (18). A study showed that defective IFN-γ pathway cannot be the only cause of clinical TB in most cases. This study shows that PBMCs in clinical TB patients had normal responsiveness to IFN-γ and that no known genetic mutations were present in the IFN-γR1 and STAT1 genes (17).

The prognosis of patients with IFN-γR1 deficiency is poor and IFN-γ therapy is not effective because of lack of functional receptors (19, 21). Bone marrow transplantation is the only effective treatment for children suffering from complete IFN-γR deficiency (35). In our study, no relationship was observed between IFN-γ receptor and lymphadenopathy. The results of various

studies should also be compared taking into account the measurement methods used. In this study ELISA was used for IFN-γ and flow cytometry for IFNγ-R. Despite numerous studies, because the underlying cause of the development of this condition is unknown in some people, careful examination of immune responses greatly assists in finding out the cause of its complications. Given the substantial role of the immune system in the development of complications after BCG vaccination, especially severe axillary lymphadenitis, and that BCG is injected at advanced ages, family history of immunodeficiency should also be taken into account. If the immune system of the infant is weak, the vaccination could be delayed (36).

4-1. Limitations of the study

The sample size was relatively low due to the small number of individuals who enrolled in the case group and sampling from different populations is preferable.

5- CONCLUSION

IFN-γ level was significantly lower in the BCG lymphadenopathy group than in the control group, indicating the role of this cytokine in developing this disease. In BCG vaccine induced axillary lymphadenopathy, it seems that the role of IFN-γ in interacting with other cytokines and other immune cells should be investigated. The levels of IFN-γR (CD119 level in PBMC) in the two groups did not show a significant relationship, which could be due to the rare incidence of IFN-γR deficiency in the studied population.

6- ACKNOWLEDGEMENT

This study was obtained from a research project approved by Shahrekord University of Medical Sciences. Hereby, the financial support of the University (grant no.: 2528), and the cooperation of Imam Ali Clinic staff are acknowledged.

7- AUTHORS' CONTRIBUTIONS

AKh: Study conception, sample collection; MAZ: Sample collection; AAR: Sample collection; SKh: Data analysis; KGh: CD119 and IFN-γ experimentations; HS: Sample collection; NKh: Study conception, manuscript edition.

8- CONFLICT OF INTEREST: None.

9- REFERENCES

1. Manju V, Parida A, Hegde A, Kousikakatakam P. Upsurge in BCG vaccine induced lymphadenitis: Case series. J Young Pharm; 2018; 10(1):123-25.

2. Noorbakhsh S, Mousavi J, Barati M, Shamshiri AR, Shekarabi M, Tabatabaei A, Soleimani Gh. Evaluation of an interferon-gamma release assay in young contacts of active tuberculosis cases. Eastern Mediterranean Health Journal.2011; 9: 714-18.

3. Shirvani F, Karimi A, Rajabnejad M. BCG vaccination as a prevention strategy, threats and benefits. Arch Pediatr Infect Dis. 2016; 4(2):e30180.

4. Sulis G, Roggi A, Matteelli A, Raviglione MC. Tuberculosis: epidemiology and control. Mediterr J Hematol Infect Dis. 2014; 6(1):e2014070.

5. Pendharkar D, Hassan MJ, Khan S, Khetrapal S, Ahmad N, Jetley S. Cytological diagnosis and management of Bacille-Calmette-Guerin (BCG) induced lymphadenitis in infants. Indian J PatholOncol. 2019; 6(1):63-66.

6. Norouzi S, Aghamohammadi A, Mamishi S, Rosenzweig SD, Rezaei N. Bacillus Calmette-Guerin (BCG) complications associated with primary immunodeficiency diseases. J Infect. 2012; 64(6):543-554.

7. Kashyap RS, Husain AA, Morey SH, Panchbhai MS, Deshpande PS, Purohit HJ, et al. Assessment of immune response to repeat stimulation with BCG vaccine using in vitro PBMC model. J Immune Based Ther Vaccines. 2010; 8: 3.

8. Sadeghi-Shanbestari M, Ansarin K, Maljaei SH, Rafeey M, Pezeshki Z, Kousha A, et al.

Immunologic aspects of patients with disseminated bacilleCalmette-Guerin disease in north-west of Iran. Ital J Pediatr. 2009 Dec 23; 35: 42.

9. Behjati M, Ayatollahi J. Post BCG lymphadenitis in vaccinated infants in Yazd, Iran. Iran J Pediatr, 2008: 18(4): 351-56.

10.Caglayan S, Yegin O, Kayran K, Timocin N, Kasirga E, Gun M. Is medical therapy effective for regional lymphadenitis following BCG vaccination? Am J Dis Child. 1987; 141(11): 1213-14.

11. Romero-Adrian TB, Leal-Montiel J, Fernández G, Valecillo A. Role of cytokines and other factors involved in the Mycobacterium tuberculosis infection. World J Immunol. 2015; 5(1):16-50.

12. Schroder K, Hertzog PJ, Ravasi T, Hume DA. Interferon-gamma: an overview of signals, mechanisms and functions. J Leukoc Biol. 2004; 75(2):163-89.

13. Hussain S, Afzal N, Javaid K, Ullah MI, Ahmad T, SaleemUz Z. Level of interferon gamma in the blood of tuberculosis patients. Iran J Immunol. 2010; 7(4):140-46.

14. Chan W, Kwan Y, Leung C. Management of Bacillus Calmette-Guérin Lymphadenitis. Hong Kong J Paediatr. 2011; 16(2): 85-94.

15. Novick D, Orchansky P, Revel M, Rubinstein M. The human interferon-gamma receptor. Purification, characterization, and preparation of antibodies. J Biol Chem. 1987; 262(18):8483-87.

16. Flynn JL, Chan J, Triebold KJ, Dalton DK, Stewart TA, Bloom BR. An essential role for interferon gamma in resistance to Mycobacterium tuberculosis infection. J Exp Med. 1993; 178(6):2249-54.

17. Park GY, Im YH, Ahn CH, Park JW, Jeong SW, Ahn JY, et al. Functional and genetic assessment of IFN-gamma receptor in patients with clinical tuberculosis. Int J Tuberc Lung Dis. 2004; 8(10):1221-27.

18. Farhondi A, Barzargan N, Pourpak SAZ. BCG dissemination in 40 patients and the review of" leukocyte mycobacterium defect" in one patient. Iran J Allergy Asthma Immunol, 2000; 1(2): 63-6.

19. Rezai MS, Ahangarkani F, Sadeghi R, Mahdavi MR. Evaluation of children with complication of BCG vaccination in north of Iran. Int J Pediatr 2017; 5(3): 4479-88.

20. Beshir MR, Zidan AE, El-Saadny HF, Ramadan RA, Karam NA, Amin EK, et al. Evaluation of the Immune Response to Interferon Gamma Release Assay and Tuberculin Skin Test Among BCG Vaccinated Children in East of Egypt: A Cross-Sectional Study. Medicine (Baltimore). 2016; 95(17):e3470.

21. Shahmohammadi S, Saffar MJ, Rezai MS. BCG-osis after BCG vaccination in immunocompromised children: Case series and review. J Pediatr Rev. 2014; 2(1):62-74.

22. Rezai MS, Khotaei G, Mamishi S, Kheirkhah M, Parvaneh N. Disseminated Bacillus Calmette-Guerin infection after BCG vaccination. J Trop Pediatr. 2008; 54(6):413-16.

23. Goraya JS, Virdi VS. BacilleCalmette-Guerin lymphadenitis. Postgrad Med J. 2002; 78(920):327-29.

24. Issa AH, Salman MS. Polymorphism of interferon gamma promoter and receptor among tuberculosis patients in Basra province, south of Iraq. Donnish J Med, Med Sci. 2017; 4(1):1-7.

25. Boskovska K, Naceva-Fustic S, Simonovska L, Dilberovska M, Dacevski D, Popova G, et al. Comparison of IFN-gamma Levels in Children with Tuberculosis Disease (TB) and Latent Tuberculosis Infection (LTBI). Open Access Maced J Med Sci. 2018; 6(11):2091-96.

26. Li Q, Li J, Tian J, Zhu B, Zhang Y, Yang K, et al. IL-17 and IFN-gamma production in peripheral blood following BCG vaccination and Mycobacterium tuberculosis infection in human. Eur Rev Med Pharmacol Sci. 2012; 16(14):2029-36.

27. Kim YK, Uh Y, Lee NS, Cho MY, Eom M, Kim HY. Whole-blood interferon-gamma release assay for diagnosis of tuberculous lymphadenitis. Tohoku J Exp Med. 2011; 224(3):189-93.

28. Kagina BM, Abel B, Scriba TJ, Hughes EJ, Keyser A, Soares A, et al. Specific

T cell frequency and cytokine expression profile do not correlate with protection against tuberculosis after bacillus Calmette-Guerin vaccination of newborns. Am J RespirCrit Care Med. 2010; 182(8):1073-79.

29. Vordermeier HM, Chambers MA, Cockle PJ, Whelan AO, Simmons J, Hewinson RG. Correlation of ESAT-6-specific gammainterferon production with pathology in cattle following Mycobacterium bovis BCG vaccination against experimental bovine tuberculosis. Infect Immun. 2002; 70(6):3026-32.

30. Condos R, Raju B, Canova A, Zhao BY, Weiden M, Rom WN, et al. Recombinant gamma interferon stimulates signal transduction and gene expression in alveolar macrophages in vitro and in tuberculosis patients. Infect Immun. 2003; 71(4):2058-2064.

31. Liu Q, Li W, Chen Y, Du X, Wang C, Liang B, et al. Performance of interferon-gamma release assay in the diagnosis of tuberculous lymphadenitis: a meta-analysis. PeerJ. 2017; 5: e3136.

32. Pai M, Dheda K, Cunningham J, Scano F, O'Brien R. T-cell assays for the diagnosis of latent tuberculosis infection: moving the research agenda forward. Lancet Infect Dis. 2007; 7(6):428-38. PubMed PMID: 17521596.

33. Gudjonsdottir MJ, Kotz K, Nielsen RS, Wilmar P, Olausson S, Wallmyr D, et al. Relation between BCG vaccine scar and an interferon-gamma release assay in immigrant children with "positive" tuberculin skin test (>/=10 mm). BMC Infect Dis. 2016; 16(1):540.

34. Pourakbari B, HosseinpourSadeghi R, Mahmoudi S, Parvaneh N, KeshavarzValian S, Mamishi S. Evaluation of interleukin-12 receptor beta1 and interferon gamma receptor 1 deficiency in patients with disseminated BCG infection. AllergolImmunopathol (Madr). 2019; 47(1):38-42.

35. Sundaram B, Amperayani S, Dhanalakshmi K, Padmanaban S. Gamma interferon receptor defect presenting as recurrent tuberculosis. Indian J Pediatr. 2014; 81(7):696-98.

36. Santos A, Dias A, Cordeiro A, Cordinha C, Lemos S, Rocha G, et al. Severe axillary lymphadenitis after BCG vaccination: alert for primary immunodeficiencies. J MicrobiolImmunol Infect. 2010; 43(6):530-37.