

## BCG Vaccine: Necessary or Not for North Cyprus

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### ABSTRACT

The cross-sectional study was performed 2016 to 2020 in North Cyprus. A total of 288 patients were studied with TB culture, Ziehl-Neelsen Staining, PCR and the QuantiFERON TB Gold Plus. SPSS was used for the statistical analysis. The number of patients who found as positive for any of the samples were 27 (9.4%). Considering the distribution over the years, it was seen that TB positive patients increased significantly between 2016 and 2020 ( $p=0.000$ ). It is seen that the prevalence of TB among foreign nationals is higher than that of other nationals ( $p=0.042$ ). Also, It has been observed that TB positivity among North Cyprus citizens has increased over the years. However, this increase is not statistically significant ( $p=0.062$ ). Our results indicated that the higher TB positivity was found in other nations (18.8%) and the TB positivity among North Cyprus citizens has increased over the years. So that, we suggested that the question of ‘BCG vaccine is necessary or not’ might be revisited by the Ministry of the Health according to our results.

**Keywords:** Tuberculosis, *Mycobacterium tuberculosis*, Vaccine, North Cyprus

### Introduction

Tuberculosis (TB) is a chronic infectious disease caused by the bacterium called *Mycobacterium tuberculosis* (*M. tuberculosis*), which can infect humans and animals and is one of the leading causes of morbidity and mortality worldwide. Pulmonary tuberculosis is seen primarily in patients but it can also cause extra pulmonary tuberculosis by infecting bones, cerebrospinal membrane, joints, intestines, skin, lymph nodes, kidneys and other organs (1). According to the data of the World Health Organization (WHO), a total of 10 million people, including 5.6 million men, 3.2 million women and 1.2 million children got TB in 2019. One million four hundred of these patients (208,000 of them HIV positive) died due to TB infection. (2).

BCG (Bacillus Calmette-Guerin) is a tuberculosis vaccine discovered in 1908, which is obtained from attenuated strain of *Mycobacterium bovis* (*M.bovis*). It is an attenuated mycobacterial *M. bovis* strains that contains all the structural features of the tuberculosis bacillus but not likely to cause TB. It activates T lymphocytes and macrophages as in normal infection and provides an immune response against tuberculosis (3). BCG is the only vaccine included in WHO's Extended Immunization Program and currently valid against TB (4). BCG vaccine first applied in 1921 to humans and also widely used in Turkey since 1951, which became a part of the National Immunization Calendar of Turkey. It is performed in the pediatric age group in the second month to develop stronger immunization (5).

Cyprus is a third largest country in the Eastern Mediterranean and located south of Turkey, west of Syria and Lebanon, North of Egypt, Israel and southeast of Greece. The North part of the Cyprus is consist of approximately 375 000 population whome are Turkish Cypriots (TRNC State Planning Organization). In North Cyprus, Tuberculin Skin Test (PPD/Purified Protein Derivative) is performed to detect and to start the treatment, instead of vaccines in the fight against TB. Therefore PPD test is regularly applied to primary school first and fifth grades every year. In addition, it is compulsory to have PPD test to those who come to our country from abroad and want to get a job, residence and student permission (6).

To our knowledge, no study has ever investigated these parameters in the North Cyprus to date. Therefore, The aim of this study is to determine the prevalence of TB by evaluating the samples of patients coming to the Near East University Hospital microbiology laboratory, which is the only mycobacteriology laboratory in North Cyprus. In addition, it is among our goals to discuss the necessity of the BCG vaccine, which is not included in the routine vaccination calendar of North Cyprus.

## Methods

### Study group

This cross-sectional study was carried out during 2016 to 2020. Samples were analyzed in a microbiology laboratory at Near East Hospital, in North Cyprus. All the suspected cases of *Mycobacterium tuberculosis* infection visiting the microbiology laboratory of the hospital were enrolled, and informed consent was obtained from the patients. Samples were obtained from patients in different hospital wards. Samples that were not suitable for transfer or not approved by the center expert were excluded from the study.

### Tuberculosis culture

N-acetyl-cysteine - 4% NaOH - 2.9% sodium citrate (NALC-NaOH) method was used for the decontamination and homogenization of the samples. 5-10 ml of each sample was mixed with an equal amount of NALC-NaOH solution and the mixture was mixed with a vortex mixer for not more than 30 seconds. Tubes were kept at room temperature for 15 minutes and shake. Phosphate buffer (0.067 M, pH = 6.8) was added up to 50 ml of the mixture, the sediment obtained by centrifugation at 3000Xg for 15 minutes was diluted with 1-2 ml of phosphate buffer (pH = 6.8). A preparation was prepared from this solution for dyeing. 0.5 ml of the prepared clinical samples

were taken and cultivated on Bactec Mycobacterium Growth Indicator Tube (MGIT-960, BD, Biosciences, Sparks, MD) medium and to the Lowenstein Jensen (Invitro Diagnostic Becton Dickinson and Company, USA) medium. MGIT tubes were kept in BACTEC MGIT-960 device at 37 ° C for 6-8 weeks, Lowenstein Jensen (LJ) media were kept in a laboratory incubator at the same temperature and time. At the end of this period, the result was reported as negative for non-reproductive samples.

**Ziehl-Neelsen (Acid Fast) Staining:** Prepared samples smear on a clean sterile microscopic slide fixed by heat. Carbol fuschin applied over the smear and heated gently until steam rises around 5 minutes. The smear slide rinsed well until no colour appears in the water. The smear slide then covered with 3% v/v acid alcohol. Washed well with clean water and covered the smear with methylene blue for 30 seconds. The microscopic examination was done under the 100x objective of the light microscope.

**Polimerase Chain Reaction:** DNA was extracted from clinical specimens with an RTP Mycobacteria Kit (STRATEC Molecular GmbH, BerlinBuch, Germany) according to the manufacturer's instructions. Briefly, 200 µl of the clinical specimen was mixed with 200 µl of NAC buffer, kept at room temperature for 20 min, and centrifuged at 11,000 x g. The supernatant was then mixed with 400 µl of buffer R and transferred to an extraction tube. Other steps were performed according to the standard protocol. The extracted DNA purity and concentration were determined on a Nanodrop 1000 (Thermo Scientific). Samples were stored at -20°C until further analyses. The PCR protocol was as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 45 sec, amplification at 63°C for 30 sec, and extension at 72°C for 45 sec, followed by a final extension at 72°C for 5 min. The PCR products were electrophoresed on 1.5% agarose gels.

**The QuantiFERON-TB Gold Plus:** The QFT-Plus kits were donated by Qiagen and were used according to the manufacturer's instructions (Quiagen,2015). The results of the QFT-Plus assay were defined as positive if either or both of the TB antigen tubes (TB1 and/or TB2) were positive (Thell ES, 2018). If antigen-nil was 8 IU/mL or (2) antigen-nil 0.35 IU/mL and 10 IU/mL were classified as 10 IU/mL.

**Statistical analysis:** SPSS Demo Ver 22.0 (SPSS Inc., Chicago, IL, USA) program was used for the statistical analysis. Person Chi-Square was used to determine the statistical significance. The significance was considered when  $p < 0.05$ .

**Ethical approval:** The study was approved by Near East University Institutional Review Board (Project no: NEU/2020/85-1197).

## Results

Total patient number were 288 (Male: 162, 56.3%; Female: 126, 43.8%) and the mean age was  $47.17 \pm 22.98$  (0-95 age). 207 (71.9%) samples were sputum, 16 (5.6%) samples were

aspiration fluid, 27 (9.4%) samples were urine, 33 (11.5%) samples were blood and 5 samples (1.7%) were cerebrospinal fluid.

The number of patients who found as positive for any of the samples were 27 (9.4%). The mean age was  $44.85 \pm 20.75$  (10-82 ages). Eighteen (66.7%) of these TB positive patients were male and 9 (33.3%) were female. There is no statistically significant difference between TB positivity and the gender ( $p=0.252$ ).

Departments that patients have applied are analysed and found that; 124 (43.1%) patients were from department of chest diseases and allergy, 60 (20.8%) patients were from department of infectious diseases and clinical microbiology, 35 (12.2%) patients were from department of internal medicine, 16 (5.6%) patients were department of pediatric, 12 (4.2%) patients were department of cardiology, 8 (2.8%) patients from department of emergency service, 7 (2.4%) patients were from department of neurology services, 7 (2.4%) patients were from department of neurosurgery, 6 (2.1%) patients were from department of urology, 6 (2.1%) patients were from intensive care unit, 3 (1.0%) patients form department of otolaryngology, 2 (0.7%) patients from department of general surgery and 2 (0.7%) patients were from department of oncology.

One hundred forty two samples analyzed only Ziehl-Neelsen (Acid Fast) Staining; 10 samples (3.5%) were found positive whereas 132 (45.8%) samples were found negative. One hundred three samples analyzed only TB culture; 7 (2.4%) samples were found positive whereas 96 (33.3%) samples were found negative. Thity three samples analyzed only for QFT-Plus; 8 (2.8%) samples were found positive whereas 25 (8.7%) samples were found negative. Twenty samples analyzed only for PCR; 2 (0.7%) samples were found positive and the 18 (6.3%) samples were negative.

Considering the distribution over the years, it was seen that TB positive patients increased significantly between 2016 and 2020 ( $p=0.000$ ) (Figure 1). Positive number of patients by years reported as; 0 patients in 2016, 4 patients in 2017, 1 patients in 2018, 10 patients in 2019 and 12 patients in 2020. Considering the distribution over the years, it was seen that TB positive patients increased significantly between 2016 and 2020 ( $p=0.000$ ) (Figure 1).

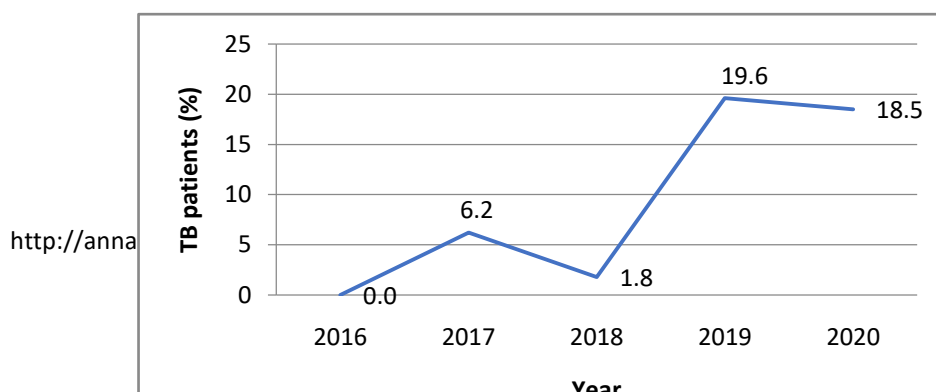


Figure1. Distribution of TB positive patients over the year

Patients resulted as TB positive also analysed according to the nationalities. 14 (8.2%) TB positive patients were from North Cyprus, 4 (5.7%) TB positive patients were from Turkey and 9 (18.8%) TB positive patients were from the other nations. A statistically significant difference was found between TB positivity and nationalities. Accordingly, it is seen that the prevalence of TB among foreign nationals is higher than that of other nationals ( $p=0.042$ ). Also, It has been observed that TB positivity among North Cyprus citizens has increased over the years. However, this increase is not statistically significant ( $p=0.062$ ) (Figure 2).

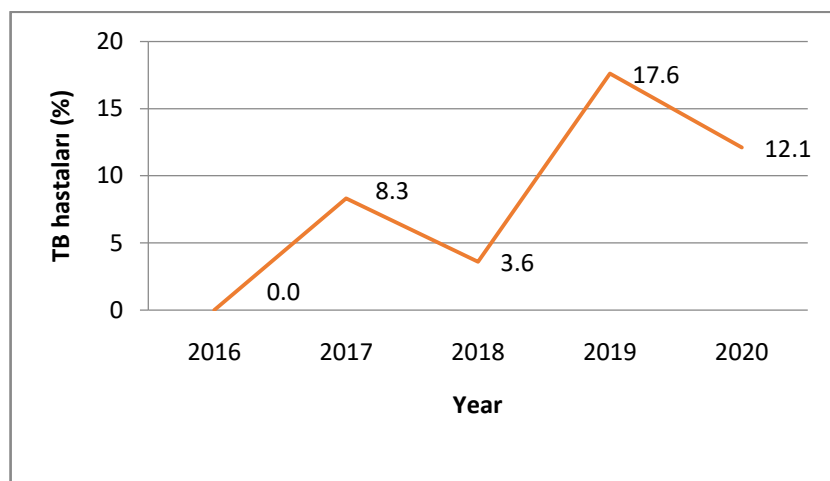


Figure 2. Distribution of TB positive patients according to years in North Cyprus

## Discussions

Our study aimed at estimating TB prevalence in the population of North Cyprus and investigating BCG vaccine added in the North Cyprus vaccination calendar. The sample included 288 patients, mostly men (56.3 %). Similar to other studies carried out in Turkey and the world, this study found a higher proportion of males than females among TB patients (7-10). However, there is no statistically significant difference between TB positivity and the gender

The another indicator factor of the TB's epidemiology is the age distribution of tuberculosis patients. Therefore, tuberculosis is most common among the elderly and mostly results from the reactivation of a previous primary infection şn developed countries however TB

affects all age groups, especially youth and young adults in developing countries (11-13). In our study, although the majority of the patients were in the 10- 82 year age group like other developing countries (8).

According to the Global Tuberculosis Report 2020, The prevalence of the South-East Asia was 44%, Africa was 25% and the Western Pacific was 18%, with smaller percentages in the Eastern Mediterranean was 8.2%, the Americas was 2.9% and Europe was 2.5%. Also, Voniatis C et al reported that South part of the Cyprus has virtually reached the elimination phase in its native born population (14). In our study, the prevalence of the North Cyprus was 9.4%. However, the prevalence of the South part of the Cyprus was 5.3% in 2019 (15).

North Cyprus is one of the destinations for casino tourism and entertainment, alongside to its sunny beaches and the island of the university campus. Therefore, lots of the other nationalities live in North Cyprus. According to our study, The percentage of the TB positive patients from North Cyprus was 8.2%; TB positive patients from Turkey was 5.7% and TB positive patients from other nations were 18.8%. There was the statistically significant difference was found between TB positivity and nationalities ( $p=0.042$ ).

### Conclusion

The North Cyprus has a TB control programme. Since 1980, all country has been screening with PPD, the Tuberculin Skin Test organization by The Ministry of Health, North Cyprus. Therefore, TB is not a public health problem. Also, TB positive patients are identified by the PPD test instead of BCG vaccine because of the side effects of the BCG vaccine and protection of the vaccine is not desired level. Therefore, BCG vaccine has not been in North Cyprus vaccine calendar. However, in North Cyprus population have been changed with globalization such as the tourist and the university student number has been increasing every year. Additionally, the people from different countries started to be included in the North Cyprus population and diversity started to increase. Our results indicated that the higher TB positivity was found in other nations (18.8%) and the TB positivity among North Cyprus citizens has increased over the years. So that, we suggested that the question of 'BCG vaccine is necessary or not' might be revisited by the Ministry of the Health according to our results

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