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TANAFFOS

Relationship between Tuberculin Skin Test and COVID-19 Outcomes among Patients with COVID-19 in Zahedan, Iran

Roya Alavi-Naini [1], Narjes Sargolzaie [1,2], Maliheh Metanat [1]

[1] Infectious Diseases and Tropical Medicine Research Center, Resistant Tuberculosis Institute, Zahedan University of Medical Sciences, Zahedan, Iran, [2] Clinical Immunology Research Center, Zahedan University of Medical Sciences, Zahedan, Iran.

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Correspondence to: Sargolzaie N
Address: Infectious Diseases and Tropical Medicine Research Center, Resistant Tuberculosis Institute, Zahedan University of Medical Sciences, Zahedan, Iran
Email address: sargolzaeinarges@gmail.com

Background: Ecological studies showed that countries with national Bacillus Calmette-Guerin (BCG) vaccination programs for tuberculosis prevention reported lower incidences of severe and fatal COVID-19 than countries without such programs. Several studies have demonstrated that the BCG vaccine can induce long-term trained Immunity in bone marrow progenitor cells. In this study, we tried to evaluate the relationship between tuberculin skin test results, BCG scar, and COVID-19 outcomes among patients with confirmed COVID-19.

Materials and Methods: This was a cross-sectional study. Cases included 160 patients with confirmed COVID-19 in Zahedan hospitals (southeast Iran) in 2020, selected by convenient sampling. PPD test was performed for all patients through the intradermal technique. Collected data included demographic information, underlying conditions, PPD test results, and COVID-19 outcome. Analysis was conducted utilizing ANOVA, $\chi 2$ test, and multivariate analysis (logistic regression).

Results: The univariate analysis showed a positive relationship between older age, having underlying diseases, and positive tuberculin skin test results with the outcome of COVID-19. We also found a lower frequency of BCG scar among patients with death outcomes than recovered ones. In the multivariate analysis by logistic regression through the backward method, only age and underlying diseases remained predictors of death.

Conclusion: Tuberculin test results might be dependent on age and underlying conditions. Our study did not show relationship between BCG vaccine and mortality in COVID-19 patients. Further investigations in different settings are required to reveal the efficacy of the BCG vaccine in preventing this devastating disease.

Key words: COVID-19; Tuberculin Skin Test; Purified protein derivative; BCG vaccine

INTRODUCTION

Globally, up to 3 September 2021, there were 218,946,836 confirmed reported cases of COVID-19, including 4,539,723 deaths, to the WHO (1). Significant mortality due to severe pneumonia with ARDS and respiratory insufficiency, particularly in older patients, has made COVID-19 the most critical global health challenge since December 2019. Numerous efforts have been made to prevent and treat this novel respiratory tract infection. Only an effective vaccine can prevent the spread of the virus .Ecological studies have demonstrated that countries with national Bacillus Calmette-Guerin (BCG) vaccination programs for tuberculosis (TB) prevention have reported

lower rates of fatal and severe COVID-19 than countries without such programs (2). BCG vaccine strengthens the human immune system against several types of viruses, including human respiratory syncytial Virus (hRSV) and human papillomavirus (HPV). Current evidence indicates that the BCG vaccine can prevent viral infections by fostering immune responses against viruses (3). The World Health Organization (WHO) recommends BCG vaccination (one dose) in all neonates of countries with a high incidence of tuberculosis (TB) (4). Long-term protection of the BCG vaccine has been reported in controlled trials up to approximately 60 years after vaccination (5).

Specific microbial contacts can activate long-lasting epigenetic changes in innate immune cells. This immunity not only enhances later response to a second infection with the same microbe but also to other microbial insults. Furthermore, trained immunity or innate immune memory has been documented for the BCG vaccine. Activation of the Immune memory can enhance responsiveness to subsequent triggers and is called trained immunity (6).

Several studies have demonstrated that the BCG vaccine can induce long-term trained Immunity in bone marrow progenitor cells through epigenetics changes (6, 7). Tuberculosis skin test with purified protein derivative (PPD) skin test, through the Mantoux technique, is a type IV hypersensitivity skin reaction to the tuberculin antigen. Koch developed this test through the intradermal technique and was further developed by Charles Mantoux in 1912. A tuberculin skin test is interpreted by measuring the hypersensitivity reaction (delayed-type hypersensitivity) to tuberculin purified protein derivative derived from Mycobacterium tuberculosis. Cell infiltration makes induration of the skin at the injection site. Reading should be done 48 to 72 hours after the test (8).

In this study, we tried to evaluate the relationship between tuberculin skin test results, BCG scar, and outcomes among patients with confirmed COVID-19 in Zahedan, southeast Iran.

MATERIALS AND METHODS

Study design and subjects

This was a cross-sectional study. Cases included 160 patients with confirmed COVID-19 in Zahedan hospitals in 2020 selected by convenience sampling. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection was confirmed through nasopharyngeal polymerase chain reaction (PCR) test in all cases. After explaining the research purpose and obtaining Informed consent, the PPD test was performed through the intradermal technique (Mantoux technique). The standard recommended tuberculin test was administered by injecting 0.1 mL of a liquid containing 5 TU (tuberculin units) of PPD into the top layers of the skin (intradermal) of the forearm. Results were read 48 hours later by measuring the skin induration diameter. Patients were followed until the outcomes of recovery or death. COVID-19 outcome was recorded in four groups: 1) outpatient recovery, 2) recovery after hospitalization without intensive care need, 3) recovery after intensive care, and 4) death.

Data collection

Data checklists were completed for patients that included demographic data, underlying conditions (hypertension, cardiovascular disease, diabetes, chronic lung diseases, immunocompromising conditions, chronic kidney diseases, and obesity), PPD test results, and COVID-19 outcomes.

Statistical analyses

Continuous and categorical data were expressed as mean ± SD (standard deviation) and number (%). The relationship between skin PPD test results, BCG scars, and COVID-19 outcomes were analyzed using ANOVA and χ^2 tests. Multivariate analysis (logistic regression) was conducted to determine the predicting effect of different variables on the death outcome. A P-value of <0.05 was considered statistically significant.

RESULTS

This study was performed on 160 patients with confirmed COVID-19 infection, including 93 males and 67 females. The mean age of the patients was 49.2 ±13.5 years. The frequencies of different outcomes were as follows: 52 (32.5%) outpatient cases recovered completely, 78 (48.7%) patients recovered after hospitalization without intensive care need, 10 (6.3%) patients recovered after intensive care, and 20 (12.5%) cases perished.

Table 1 shows the statistical differences in the mean ages between different outcomes of COVID-19. Pearson correlation analysis showed a positive correlation between the severity of COVID-19 and age (r=0.401, P<0.001). The frequency of underlying diseases in patients who died was significantly higher than in those who recovered (P<0.001) (Table 2).

Tuberculin skin test results and the frequency of BCG scar among the study population with COVID-19 infection are summarized in Table 3. The study's results revealed that the rate of positive tuberculin skin test among the deceased patients was significantly greater compared to other groups (P<0.001). Moreover, as seen in the table, the frequency of BCG scar was statistically lower in patients with death outcomes compared with the recovered ones (P< 0.001).

In addition, quantitative analysis (Table 4) indicated that the results of the tuberculin skin test (the mean diameter of the skin induration) in patients with a death outcome were significantly higher compared to the other groups (P= 0.020). The results of the multivariate analysis (logistic regression through the backward method) to determine the predicting effect of different variables on death are summarized in Table 5. This table shows four backward steps to assess the predictive effects of different variables and demonstrates that only age and underlying diseases remained predictors of death.

Table 1. Comparison of mean age between three groups of patients

Groups	N %	Mean	Std. Deviation	95% Confidence Interval for Mean		P value
Outpatient recovery	52	41.74	9.06	39.16	44.31	
Recovery after hospitalization without intensive care need and Recovery after intensive care	88	50.76	13.57	47.88	53.63	
Death	20	60.65	12.43	54.82	66.47	0.007
Total	160	49.15	13.50	47.03	51.27	

Table 2. Comparison of the frequency of underlying diseases between three groups of patients

Groups	Underlying Diseases			P value
	N	No Count (%)	Yes Count (%)	
Outpatient recovery	52	46(88.5)	6 (11.5)	
Recovery after hospitalization without intensive care need and Recovery after intensive care	88	35 (39.8)	53 (60.2)	
Death	20	1 (5)	19 (95)	<0.001
Total	160	82 (51.2)	78 (48.8)	

Table 3. Tuberculin skin test results and BCG Scar among studied patients with COVID-19 infection

Groups	Tuberculin skin test results		P Value	BCG Scar		P Value
	Positive Number (%)	Negative Number (%)		Positive Number (%)	Negative Number (%)	
Outpatient recovery (group1)	6 (11.5)	46 (88.5)		51 (98)	1 (2)	
Recovery after hospitalization without intensive care need and Recovery after intensive care (combined group2 and group3)	26 (29.5)	62 (70.5)	<0.001	67 (76.1)	21 (23.9)	<0.001
Death (group 4)	14 (70)	6 (30)		11 (55)	9 (45)	
Total	46 (28.7)	114 (71.3)		129 (80.5)	31 (19.5)	

*: Induration more than 10 millimeter

Table 4. Quantitative results of tuberculin skin test among studied patients with COVID-19

Groups	Number	Mean (mm)	Std. Deviation	Minimum	Maximum	P Value
Outpatient recovery	52	3.13	4.16	0	18	
Recovery after hospitalization without intensive care need and Recovery after intensive care	88	4.55	4.63	0	20	0.020
Death	20	6.35	4.60	0	17	

Table 5. Logistic regression analysis; prediction effect of different variables for death in COVID-19 patients

		B	S.E.	Wald	P Value	Odds ratio
Step 1	Age	0.052	0.02	4.46	0.035	1.05
	Gender	-0.72	0.57	1.57	0.209	0.48
	PPD induration	0.08	0.05	2.17	0.140	1.08
	BCG Scar	0.45	0.59	0.58	0.446	1.57
	Underlying Diseases	2.53	1.07	5.54	0.019	12.61
	Constant	-6.72	1.69	15.67	0.00	0.00
Step 2	Age	0.05	0.02	6.41	0.01	1.06
	Gender	-0.75	0.57	1.74	0.18	0.46
	PPD induration	0.09	0.05	2.62	0.10	1.09
	Underlying Diseases	2.59	1.07	5.85	0.01	13.39
	Constant	-6.51	1.67	15.17	0.00	0.00
Step 3	Age	0.05	0.02	5.62	0.01	1.05
	PPD induration	0.08	0.05	2.42	0.11	1.09
	Underlying Diseases	2.48	1.07	5.38	0.02	12.03
	Constant	-7.18	1.59	20.38	0.00	0.00
Step 4	Age	0.04	0.02	4.39	0.03	1.04
	Underlying Diseases	2.69	1.06	6.34	0.01	14.79
	Constant	-6.40	1.44	19.58	0.00	0.00

DISCUSSION

In univariate analysis, our results showed a positive relationship between older age, having an underlying disease, positive tuberculin skin test results, and the outcome of COVID-19. We also found a lower frequency of BCG scars among patients with death outcomes than in other groups of recovered patients. Many studies have revealed that age and underlying diseases are risk factors for COVID-19 disease severity, ICU admission, and death (9-14). We investigated whether PPD test results correlated with the outcomes of patients with COVID-19. Cell-mediated immunity has a prominent role in protecting against certain viral infections (15), and T cells are the primary immune cells that react against viral infections (16). Gamma interferon (IFN-γ) released by macrophages and cytokines produced by antigen-specific T cells are the primary immune responses against *M. tuberculosis* (17, 18).

A significant correlation has been reported between IFN-γ and the PPD-Skin test (19, 20). Several studies have reported the activation of host immune system as Th1 and Th2 response in COVID-19 and concluded that higher IFN-γ concentrations might be related to the ultimate discharge or death outcomes in patients with COVID-19 (21).

In our study, the univariate analysis demonstrated a relationship between COVID-19 outcomes and PPD skin test indurations which is consistent with the above studies. The innate immune memory is not specific, and infection with one infectious agent often can confer protection against other microorganisms. Similarly, the nonspecific immunity following BCG vaccination might have protective effects against several other infections, such as *C. albicans*, *S. aureus*, human respiratory syncytial virus (hRSV), and human papillomavirus (HPV) (2, 22-27).

BCG is one of the most well-known vaccines that can induce heterologous protection against other agents and diseases such as respiratory syncytial virus and yellow fever caused by RNA viruses. BCG is demonstrated to be also protective against malignancies and their recurrence (28). This non-specific effect of BCG is partially mediated via innate immune memory/trained immunity by epigenetic changes in cells of the innate and adaptive immune systems (29). Since the 1970s, in many countries with a high TB burden, the BCG vaccine has been routinely administered to newborns (30). BCG vaccine shifts lymphocyte balance towards Th1, increases gamma interferon secretion, and intensely activates regulatory T Cells (Tregs) (31).

As shown in our study, the frequency of BCG scar was statistically lower in patients who died than in all other recovered patients. Several other studies in countries with a rigorous BCG vaccination program have shown significant relationships between BCG vaccination and lower COVID-19 morbidity and mortality (25, 32-37).

In a multivariate analysis by logistic regression through the backward method, only age and underlying diseases remained predictors of death. These results are in line with the publications that concluded that tuberculin test results are dependent on age and underlying conditions. This inconsistency between our results (multivariate analysis) and the above studies might be due to ecological fallacy. Most of the mentioned studies are ecological, and associations between individual exposure and outcome are likely to be confounded by unmeasured variables (38). Measures in ecological studies are aggregated of population-level data because; societies are studied instead of individuals. The results of these studies are confounded (called ecological fallacy), which happens when relationships determined at a group level are assumed to be true in individuals (39).

The finding that patients who died were older on average is both considerable and exciting. Moreover, this group of patients was more likely to have hypertension, diabetes, cardiovascular disease, and chronic obstructive pulmonary disease. The older age and underlying conditions played significant roles in the higher mortality rate in the patients, despite the higher PPD positivity and lower rates of BCG scar in the group who did not survive.

CONCLUSION

Our study did not show relationship between BCG vaccine and mortality in COVID-19 patients. Further investigations in different settings with larger populations are required to reveal the BCG vaccine's efficacy in preventing this devastating disease.

Limitation

Some patients did not consent to PPD testing, and a group of them were not accessible for follow-up, and we had to exclude them.

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REFERENCES

1. WHO. WHO Coronavirus (COVID-19) Dashboard 2021 [Available from: https://covid19.who.int/.
2. Koti M, Morales A, Graham CH, Siemens DR. BCG vaccine and COVID-19: implications for infection prophylaxis and cancer immunotherapy. ***J Immunother Cancer*** 2020;8(2):e001119.
3. Sharma AR, Batra G, Kumar M, Mishra A, Singla R, Singh A, et al. BCG as a game-changer to prevent the infection and severity of COVID-19 pandemic? ***Allergol Immunopathol (Madr)*** 2020;48(5):507-17.
4. World Health Organization. Global tuberculosis control: surveillance, planning, financing: WHO report 2005. World Health Organization; 2005.

5. Aronson NE, Santosham M, Comstock GW, Howard RS, Moulton LH, Rhoades ER, et al. Long-term efficacy of BCG vaccine in American Indians and Alaska Natives: A 60-year follow-up study. ***JAMA*** 2004;291(17):2086-91.
6. Netea MG, Domínguez-Andrés J, Barreiro LB, Chavakis T, Divangahi M, Fuchs E, et al. Defining trained immunity and its role in health and disease. ***Nat Rev Immunol*** 2020;20(6):375-88.
7. Kleinnijenhuis J, Quintin J, Preijers F, Benn CS, Joosten LA, Jacobs C, et al. Long-lasting effects of BCG vaccination on both heterologous Th1/Th17 responses and innate trained immunity. ***J Innate Immun*** 2014;6(2):152-8.
8. Pahal P, Sharma S. PPD Skin Test (Tuberculosis Skin Test). StatPearls; StatPearls Publishing: Treasure Island, FL, USA. 2020.
9. Dudley JP, Lee NT. Disparities in Age-specific Morbidity and Mortality From SARS-CoV-2 in China and the Republic of Korea. ***Clin Infect Dis*** 2020;71(15):863-5.
10. Liang W, Liang H, Ou L, Chen B, Chen A, Li C, et al. Development and Validation of a Clinical Risk Score to Predict the Occurrence of Critical Illness in Hospitalized Patients With COVID-19. ***JAMA Intern Med*** 2020;180(8):1081-9.
11. Haybar H, Kazemnia K, Rahim F. Underlying chronic disease and COVID-19 infection: a state-of-the-art review. ***Jundishapur Journal of Chronic Disease Care*** 2020;9(2):e103452.
12. Romero Starke K, Petereit-Haack G, Schubert M, Kämpf D, Schliebner A, Hegewald J, et al. The Age-Related Risk of Severe Outcomes Due to COVID-19 Infection: A Rapid Review, Meta-Analysis, and Meta-Regression. ***Int J Environ Res Public Health*** 2020;17(16):5974.
13. Chen T, Dai Z, Mo P, Li X, Ma Z, Song S, et al. Clinical Characteristics and Outcomes of Older Patients with Coronavirus Disease 2019 (COVID-19) in Wuhan, China: A Single-Centered, Retrospective Study. ***J Gerontol A Biol Sci Med Sci*** 2020;75(9):1788-95.
14. Chen T, Wu D, Chen H, Yan W, Yang D, Chen G, et al. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. ***BMJ*** 2020;368:m1091.
15. Spencer JC, Ganguly R, Waldman RH. Nonspecific protection of mice against influenza virus infection by local or systemic immunization with Bacille Calmette-Guérin. ***J Infect Dis*** 1977;136(2):171-5.
16. Swain SL, McKinstry KK, Strutt TM. Expanding roles for $CD4^+$ T cells in immunity to viruses. ***Nat Rev Immunol*** 2012;12(2):136-48.
17. Nikitina IY, Panteleev AV, Sosunova EV, Karpina NL, Bagdasarian TR, Burmistrova IA, et al. Antigen-Specific IFN-γ Responses Correlate with the Activity of *M. tuberculosis* Infection but Are Not Associated with the Severity of Tuberculosis Disease. ***J Immunol Res*** 2016;2016:7249369.
18. Khan TA, Mazhar H, Saleha S, Tipu HN, Muhammad N, Abbas MN. Interferon-Gamma Improves Macrophages Function against M. tuberculosis in Multidrug-Resistant Tuberculosis Patients. ***Chemother Res Pract*** 2016;2016:7295390.
19. Katial RK, Hershey J, Purohit-Seth T, Belisle JT, Brennan PJ, Spencer JS, et al. Cell-mediated immune response to tuberculosis antigens: comparison of skin testing and measurement of in vitro gamma interferon production in whole-blood culture. ***Clin Diagn Lab Immunol*** 2001;8(2):339-45.
20. Pottumarthy S, Morris AJ, Harrison AC, Wells VC. Evaluation of the tuberculin gamma interferon assay: potential to replace the Mantoux skin test. ***J Clin Microbiol*** 1999;37(10):3229-32.
21. Gadotti AC, de Castro Deus M, Telles JP, Wind R, Goes M, Garcia Charello Ossoski R, et al. IFN-γ is an independent risk factor associated with mortality in patients with moderate and severe COVID-19 infection. ***Virus Res*** 2020;289:198171.
22. Sohrabi Y, Dos Santos JC, Dorenkamp M, Findeisen H, Godfrey R, Netea MG, et al. Trained immunity as a novel approach against COVID-19 with a focus on Bacillus Calmette-Guérin vaccine: mechanisms, challenges and perspectives. ***Clin Transl Immunology*** 2020;9(12):e1228.
23. Quintin J, Saeed S, Martens JHA, Giamarellos-Bourboulis EJ, Ifrim DC, Logie C, et al. Candida albicans infection affords protection against reinfection via functional reprogramming of monocytes. ***Cell Host Microbe*** 2012;12(2):223-32.
24. Arts RJ, Novakovic B, Ter Horst R, Carvalho A, Bekkering S, Lachmandas E, et al. Glutaminolysis and Fumarate

Accumulation Integrate Immunometabolic and Epigenetic Programs in Trained Immunity. ***Cell Metab*** 2016;24(6):807-19.

25. Sohrabi Y, Godfrey R, Findeisen HM. Altered Cellular Metabolism Drives Trained Immunity. ***Trends Endocrinol Metab*** 2018;29(9):602-5.
26. Kleinnijenhuis J, Quintin J, Preijers F, Joosten LA, Ifrim DC, Saeed S, et al. Bacille Calmette-Guerin induces NOD2-dependent nonspecific protection from reinfection via epigenetic reprogramming of monocytes. ***Proc Natl Acad Sci U S A*** 2012;109(43):17537-42.
27. Sharma AR, Batra G, Kumar M, Mishra A, Singla R, Singh A, et al. BCG as a game-changer to prevent the infection and severity of COVID-19 pandemic? ***Allergol Immunopathol (Madr)*** 2020;48(5):507-17.
28. O'Neill LAJ, Netea MG. BCG-induced trained immunity: can it offer protection against COVID-19? ***Nat Rev Immunol*** 2020;20(6):335-7.
29. Kleinnijenhuis J, van Crevel R, Netea MG. Trained immunity: consequences for the heterologous effects of BCG vaccination. ***Trans R Soc Trop Med Hyg*** 2015;109(1):29-35.
30. Schaaf HS, du Preez K, Kruger M, Solomons R, Taljaard JJ, Rabie H, et al. Bacille Calmette-Guérin (BCG) vaccine and the COVID-19 pandemic: responsible stewardship is needed. ***Int J Tuberc Lung Dis*** 2020;24(7):732-4.
31. Wang Z, Hong J, Sun W, Xu G, Li N, Chen X, et al. Role of IFN-gamma in induction of Foxp3 and conversion of CD4+ CD25- T cells to CD4+ Tregs. ***J Clin Invest*** 2006;116(9):2434-41.
32. Gursel M, Gursel I. Is global BCG vaccination-induced trained immunity relevant to the progression of SARS-CoV-2 pandemic? ***Allergy*** 2020;75(7):1815-9.
33. Escobar LE, Molina-Cruz A, Barillas-Mury C. BCG vaccine protection from severe coronavirus disease 2019 (COVID-19). ***Proc Natl Acad Sci U S A*** 2020;117(30):17720-6.
34. Ventura L, Vitali M, Romano Spica V. Bacillus Calmette-Guérin vaccination and socioeconomic variables vs COVID-19 global features: Clearing up a controversial issue. ***Allergy*** 2021;76(3):884-7.
35. Moorlag SJCFM, van Deuren RC, van Werkhoven CH, Jaeger M, Debisarun P, Taks E, et al. Safety and COVID-19 Symptoms in Individuals Recently Vaccinated with BCG: a Retrospective Cohort Study. ***Cell Rep Med*** 2020;1(5):100073.
36. Kinoshita M, Tanaka M. Impact of Routine Infant BCG Vaccination on COVID-19. ***J Infect*** 2020;81(4):625-33.
37. Ehtesham NZ, Samal J, Ahmad F, Arish M, Naz F, Alam A, et al. Will bacille Calmette-Guerin immunization arrest the COVID-19 pandemic? ***Indian J Med Res*** 2020;152(1 & 2):16-20.
38. Loney T, Nagelkerke NJ. The individualistic fallacy, ecological studies and instrumental variables: a causal interpretation. ***Emerg Themes Epidemiol*** 2014;11:18.
39. Munnangi S, Boktor SW. Epidemiology Of Study Design. ***StatPearls*** 2019.