Relationship between Bacillus Calmette Guerin Vaccination Policy and Coronavirus Disease-2019 (COVID-19) Incidence

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ABSTRACT

Bacillus Calmette Guerin (BCG) was designed for protecting children against tuberculosis. Also, it can protect against other infectious diseases through the induction of trained immunity. Due to its heterologous protective effects, the BCG vaccine has been proposed as a treatment option for coronavirus disease-2019 (COVID-19). Epidemiological studies have found that countries without BCG vaccination policy have experienced higher mortality rates related to COVID-19 infection than those with BCG vaccination policy. However, there are some confounding factors such as age, population intensity, immigration, the pandemic phase, and data accuracy that may affect these results. Therefore, this hypothesis should be evaluated by clinical trial studies. Large-scale clinical trials are in progress to investigate if the BCG vaccine could be used as a useful tool for protection against COVID-19 infection.

Keywords: COVID-19; Mycobacterium bovis

INTRODUCTION

Currently, the world has an encounter with coronavirus disease-2019 (COVID-19) infection caused by severe acute respiratory syndrome (SARS) coronavirus-2 (SARS-CoV-2).[1] It belongs to a positive-sense single-stranded RNA genome known as Coronaviridae family.[2] This virus induces respiratory tractinfection in human with cold, sneezing, pneumonia, and coughing, while in the animals it leads to some respiratory infection and diarrhea.[2] It can be released through droplets of saliva or from the nose of the infected person and transmitted to other individuals.[3] For the first time, it was isolated from a pneumonia patient who lives in Wuhan, China, and then spread around the world.[3] Due to rapid spread, WHO reported it as a public health emergency of international concern (PHEIC) on 30 January 2020.[4] At the time of writing, its incidence was about 3,344 per million population, with a fatality rate of 0.23 per million. Currently, there is no certain antiviral medicine or preventive vaccine for COVID-19. At the moment, treatment options for COVID-19 include drugs that have been approved for the treatment of SARS, Ebola,

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Middle East respiratory syndrome (MERS), influenza, and human immunodeficiency virus (HIV).[5] Also, six vaccines in clinical evaluation and about 70 in the pre-clinical evaluation exist for COVID-19.[6]

According to non-specific immune response created by Bacillus Calmette-Guerin (BCG) vaccination, some investigators have proposed it in COVID-19 pandemic to fight against SARS-CoV-2.[7] Epidemiological studies have been performed to investigate the BCG vaccination effects on COVID-19 incidence in countries where people have taken BCG compared to those without BCG immunization.[8-10]

BCG Vaccination and Trained Immunity

In 1921, the BCG vaccine is designed for the treatment of tuberculosis (TB) caused by *Mycobacterium tuberculosis* (Mtb) at the Institute Pasteur in Paris.BCG is a live attenuated strain obtained from *Mycobacterium bovis* isolate. Pieces of evidence showed that neonatal BCG vaccination can decrease the mortality rate in the group of children who received it and not received it.[11] Studies have shown that some BCG strains not only prevent TB but also provide effective immune responses against non-related infections and diseases.[12] A study in Guinea Bissau country, in the West-African, showed that vaccinated children experienced a 50% lower risk of overall mortality and morbidity. This effect cannot be limited to protection against tuberculosis alone but it may be related to protection against other infectious diseases such as respiratory tract infections or neonatal sepsis.[13] A study in South Africa showed that using BCG in adolescence was connected with lower respiratory tract infections.[14] Also, it has been suggested by some studies that BCG vaccine can create protection against other viral infections including Influenza, RAS, and HSV2.[15] In addition to defending against viral infections, BCG vaccination also has a protective role against yellow fever, bladder cancer, malaria, leishmaniasis, and asthma.[16-20]

It is believed that two types of immunological mechanisms mediate these beneficial effects of BCG. First, memory T cells (CD4$^+$ and CD8$^+$) have been activated by some stimuli such as cytokines in an antigen-independent manner, a process which is named heterologous immunity.[21-23] Second, BCG vaccination is associated with inducing a process known as trained immunity.[24,25] It refers to some characterizes of adaptive immune responses performed by innate immune cells like NK-cells and macrophages.[26] In this way, innate immune cells are primed through the identification of pathogen-associated molecular patterns (PAMPs) and induce a high protective inflammatory response against secondary detection of PAMPs introduced by other pathogens (cross-protection). Innate immune cells can be trained through two important mechanisms including epigenetic and metabolic reprogramming to act effectively over a second exposure.[27]

Epigenetic modification is introduced as some chemical modifications including DNA and its tone methylation, and acetylation which leads to increase chromatin accessibility, followed by easier transcription of some genes that are important in improved cell function and antimicrobial responses.[28] This mechanism does not affect the DNA sequence, but it can be transferred to the offspring. Studies have shown that the epigenetic reprogramming of monocytes at the promotor regions of inflammatory cytokine genes induced by BCG is accompanied by increased pro-inflammatory cytokines production like tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6.[29] In metabolic reprogramming certain metabolites, which have a role as co-factors for some enzymes and also have a role in the epigenetic change, are accumulated or depleted.[26] Glycolysis and glutamine metabolisms are identified as two essential factors for inducing trained immunity in monocytes by BCG vaccination.[30]

It has been revealed that some long lifespan cells such as stem cells, intestinal stromal cells, fibroblast, and microglial cells can transfer trained immunity.[26] A study has shown that BCG induces hematopoietic stem cells (HSC) and multipotent progenitors (MPPs) reprogramming in the bone marrow which leads to the generation of trained monocytes/macrophages for long-lasting immunological memory.[31] The exact duration of protection created by BCG against TB or other diseases remains unknown. Some studies have indicated that the BCG vaccine creates protection against tuberculosis for 50-60 years.[32,33] However; the exact duration of such protection against heterologous infections is unclear.

Does BCG Vaccination Policies Lead to Reduce COVID-19 Incidence?

According to these data, it has been proposed that whether BCG vaccination may lead to a protective response against COVID-19 infection and may decrease its incidence. Epidemiological studies have

shown that countries with mandated BCG vaccination have a lower number of patients with COVID-19 infection and mortality rates compared to countries without BCG vaccination.[7,8] In these epidemiological studies COVID-19 incidence and mortality rates have been compared between BCG-vaccinated countries and non-BCG vaccinated countries.

The first epidemiological study in this field was performed by Miller et al, 2020 who suggested that high rates of COVID-19 in countries such as Italy, the Netherlands, and the USA, could be related to non-universal BCG vaccination. To test this hypothesis, they evaluated and compared a large number of countries for BCG vaccination policy and COVID-19 incidence.[7] Their results showed that countries without BCG vaccination policy (Italy, Nederland, USA) had higher mortality rates than countries with mandatory BCG policy (South Korea and Japan).[7] Also, they

pointed out that countries where have adopted BCG vaccination program recently (e.g. Iran started from, 1984) had an increased mortality rate, compatible with the idea that BCG protects the vaccinated elderly population.[7] Thereafter, this hypothesis has been tested by other epidemiological studies worldwide. The results of some studies were consistent with this hypothesis while no significant correlation was seen between BCG vaccination policy and protection against COVID-19 by other studies (Table 1). Although these epidemiological data propose BCG vaccination as a protective mean against COVID-19 infection, because of several confounding factors these studies cannot be used as reliable evidence. There are some confounding factors in these epidemiological studies including differences in non-pharmaceutical interventions policy such as quarantine or social distancing, the demographic and genetic structure of the populations,

Table 1. Epidemiological studies on the association between Bacillus Calmette Guerin (BCG) vaccination and Coronavirus Disease-2019 (COVID-19) incidence

Study	Time of study	Findings
Miller et al [7]	March 21st, 2020	Decreased morbidity rate in countries with BCG vaccination policy.
Sala et al [8]	March 28st, 2020	BCG vaccination may protect SARS-CoV-2.
Berg et al [9]	April 1, 2020	A significant flattening of increase in COVID-mortality associated with BCG vaccination.
Dayal et al [10]	-	The difference in the mean CFR between vaccinated and non-vaccinated countries.
Akiyama et al [34]	April 5, 2020	A significant difference between the DT of the death between "BCG" and "non-BCG" countries.
		Using the "Tokyo 172-1" strain in BCG vaccination is associated with a longer DT of death.
Singh et al [35]	-	A negative correlation between COVID-19 morbidity and BCG vaccination.
		A positive correlation between the median age and COVID-19 morbidity.
		Religious faiths and population density of nations had not to effect on disease incidence.
Green et al [36]	December 31, 2019 to April 6, 2020	A correlation between BCG vaccination and reduced COVID-19 mortality rates.
Hegarty et al [37]	March 22, 2020	A correlation between BCG vaccination and reduced COVID-19 incidence.
Shet et al [38]	March 29, 2020	Reduced COVID-19-mortality among BCG-using countries.
Ozdemir et al [39]	April 16, 2020	The lower mean of cases per population ratio in BCG-vaccinated countries.
Singh et al [40]	April 4, 2020	Decreased the incidence rates of COVID-19 with an increase in % latent TB infection.
Aksu et al[41]	-	No association between COVID-19 incidence and BCG vaccination policy.
Hensel et al [42]	April 7, 2020	No significant association between the number of COVID-19 cases and the BCG vaccination policy.
Hamiel et al [43]	March 1 to April 5, 2020	BCG vaccination in childhood had not a protective effect against COVID-19 in adulthood.

Abbreviation: CFR=case fatality rate; DT=doubling time

Table 2. Clinical trial studies of Bacillus Calmette Guerin (BCG) forCoronavirus Disease-2019(COVID-19)

Identifier	Status	Intervention	BCG strain	Phase	Sample size	Study population	Location
NCT04534803	Not yet recruiting	BCG/Placebo	Tokyo-172	3	2100	Elderly people (70 y≤)	U.S.A
NCT04328441	Active	BCG/Placebo	Danish 1331	3	1500	HCW (18y≤)	Netherland
NCT04362124	Not yet recruiting	BCG/Placebo	BCG(freez-dried)	3	1000	HCW(18-65 y)	Colombia
NCT04379336	Recruiting	BCG/Placebo	Danish 1331	3	500	HCW (18 y≤)	South Africa
NCT 04417335	Active	BCG/Placebo	Danish 1331	4	2014	Elderly people (60 y≤)	Netherland
NCT04350931	Not yet recruiting	BCG/Placebo	Danish 1331	3	900	HCW (18 y≤)	Egypt
NCT04537663	Recruiting	BCG/Placebo	Danish 1331	4	5200	Elderly people (60y≤)	Netherland
NCT04475302	Recruiting	BCG/Control	BCG(freeze-dried)	3	1450	Elderly people (60-80 y)	India
NCT04461379	Not yet recruiting	BCG/Placebo	Tokyo-172	3	908	HCW (18 years and older)	Mexico
NCT04327206 (BRACE)	Recruiting	BCG/Placebo	Danish 1331	3	10078	HCW (18 y≤)	Australia
NCT04369794 (BATTLE)	Recruiting	BCG/Placebo	BCG (freeze-dried)	4	1000	Patient with COVID-19(18 y≤)	Brazil
NCT04414267 (ACTIVATEII)	Recruiting	BCG/Placebo	BCG (freeze-dried)	4	900	Elderly people (50 y≤)	Greece
NCT04384549	Recruiting	BCG/Placebo	Danish 1331	3	1120	HCW (18 y≤)	France
NCT04373291	Not yet recruiting	BCG/Placebo	Danish 1331	3	1500	HCW(18- l00 y)	Denmark
NCT04542330	Not yet recruiting	BCG/Placebo	Danish 1331	3	1900	Elderly people (65-110 y)	Denmark
NCT04348370 (BADAS)	Recruiting	BCG/Placebo	Tice	4	1800	HCW (18-75 y)	U.S.A
NCT04435379	Recruiting	rBCG/Placebo	VPM1002	3	2038	Elderly people (60 y≤)	Germany
NCT04439045 (COBRA)	Recruiting	rBCG/Placebo	VPM1002	3	3626	Front-line employees (18 y≤)	Canada
NCT04387409	Recruiting	rBCG/Placebo	VPM1002	3	1200	HCW (18 y≤)	Germany

Abbreviation: HCW=health care worker

case identification, population density, age distribution of population, wealth, health care system, varied BCG strains, the beginning time of study which may affect the accurate comparison of COVID-19 mortality rate between different places.

However, in some studies, the association between BCG vaccination and COVID-19 occurrence remained unchanged after adjusting for some confounding

factors. This association was seen by Berg et al after adjusting for geographic region and net migration rate, population density and size, median age,[9] and by Shet et al for the time of the epidemic, country income status, and age.[38] In addition, for mitigating the effect of such confounding factors, this hypothesis should be comprehensively tested by three approaches including meta-analysis investigations, randomized control trials, and large prospective cohort studies. Two meta-analysis studies investigated effect of BCG vaccination on the respiratory infection. A meta-analysis suggested that BCG could affect the mortality rate related to pneumonia and sepsis in children under 5 years old in low-income countries.[44] Another study reported that administration of BCG vaccine is connected with increased antibody levels against other virus vaccines such as influenza A virus and pneumococcus.[45] In addition, several clinical trial studies are ongoing to evaluate the efficacy of BCG vaccination on COVID-19 protection in some populations with a high risk of infection such as hospital care workers (HCW) or older individuals.[46] A list of active, recruiting, and not yet recruiting clinical trials for BCG vaccination was prepared in table 2.

There are about 13 active and recruiting and 5 not yet recruiting clinical trials in this field.[46] These studies are varied for BCG strain and characteristic features of the study population that can help choose an effective BCG vaccination route against COVID-19 infection. Different BCG strains including TICE (Chicago), Danish 1331 (Denmark) and, Tokyo-172 (Japan) have been used in these studies. In addition to BCG strains, two studies in Germany (NCT04435379 and NCT04387409) and one in Canada (NCT04439045) are evaluating the effect of VPM1002, a recombinant vaccine from BCG. Based on gene analysis results, the BCG vaccine has two subgroups as follows early strain, including BCGs Russia, Birkhaug, Japan, Sweden, and Moreau, and the late strains, including BCGs Danish, Glaxo, Pasteur, and Prague which are different genetically and phenotypically.[47] Some studies have reported that early and late strains have different abilities for inducing immune responses.[48] It has been shown that BCG Japan can induce more strong proliferation of T cells, higher Th1 cytokine production including IL-2, interferon (IFN)-γ, TNF-α, and IL-2, and lower Th2 cytokine production including IL-4 compared with BCG Denmark.[49] In the context of the COVID-19 pandemic, it seems that using BCG Japan

and Russia strains (early strain) are associated with a lower COVID-19 mortality rate compared with using BCG Denmark (late strain).[50] Therefore, BCG strain may affect the result of these clinical trials and should be considered in the conclusion. Another difference factor between BCG clinical trial studies is the varied characteristic features of the study population. Most studies have been performed on HCW (aged≥18 y) or elderly people (aged≥50 y) except for one study in Brazil (NCT04369794) which was performed on a patient with laboratory or clinical-epidemiological confirmation of COVID-19 (aged≥18 y). HCW and elderly individuals are more susceptible to COVID-19 infection but, selecting these groups may affect the results of the study. Due to the effects of aging on the immune system, the results of such studies can be imply assigned to old individuals, not young populations.[51] HCW have more potential risks to health in their work associated with many pathogens which may induce occupational diseases and develop abnormalities of the immune system.[52] We suppose that long-term activation of inflammatory immune responses in the HCW leads to bias in the results of the study and wrong conclusion. In addition, some clinical trials have been conducted in BCG-implemented countries such as Brazil, Colombia, Egypt, Greece, India, and Mexico. Some studies have demonstrated that BCG revaccination increases adaptive Th1, Th17, and innate effectors.[53] Therefore, the results of studies in such countries may be different from non-implemented countries. In most clinical trial studies, the sample size ranging is from 500 to 2000 except for Australia (NCT04327206) and Netherland (NCT04537663) that are about 10,000 and 5000, respectively. As less than 10 out of 1,000 individuals are estimated to be engaged with COVID-19 infection during the pandemic, such small-scale clinical trials with a small number of participants cannot be suitable for providing a clear-cut answer. Despite the defects of such clinical trial studies, those seem to be necessary for approving BCG as a protective mean for HCW and elderly people against COVID-19 until approval of a specific vaccine.

In conclusion, BCG vaccination is described to induce its heterologous protection effects through the process known as trained immunity. Some epidemiological studies have suggested that BCG-vaccinated countries have lower mortality rates due to COVID-19 compared to those without BCG

vaccination policy. However, such epidemiological studies are prone to be affected by many confounder factors, including differences in the stage of the pandemic in each country, national demographics, testing rates for COVID-19 virus infections. Therefore, this hypothesis needs to be tested by large-scale clinical trial studies. To assess BCG efficacy, some clinical trials are ongoing to investigate whether BCG vaccination could be effective for COVID-19 infection. Although current clinical trial studies in this field have some defects, its performance may be useful for confirming BCG as a suitable mean to protect especially vulnerable population such as HCW against infection with SARS-CoV-2 during current COVID-19 pandemic.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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