Review Article

COVID-19 infection: Does BCG Vaccine offers any protection

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Abstract

COVID-19 virus which is a single stranded RNA virus was responsible for infections first seen in Wuhan city in china and reached to stage of pandemic. World Health Organization (WHO) recommended use of antiviral drug therapy either alone or in combination with interferon. Interferon probably acts via alerting the cellular immune system to viral infection of host cells. Even though BCG vaccine was developed to combat tuberculosis (TB), the mortality benefit from BCG is not TB-specific but can be effective against some other organisms. Some of the unconfirmed observations and even meta-analysis of data indicate that COVID 19 pandemic is less intense in the countries with BCG vaccine intervention. Based on these data many clinical trials are being started to confirm protective effect of BCG vaccine against COVID-19 infection. However WHO stated in a press briefing that multitudes of confounding variables prevent to reach to any firm correlations. However, WHO has not ruled out the fact that the vaccine may exhibit positive effects in protecting COVID- 19 infection or reducing its severity but in absence of evidence, WHO does not recommend BCG vaccination for the prevention of COVID-19.

Keywords: BCG vaccination, COVID-19, immune system, World Health Organization

Introduction

Coronavirus Disease (COVID-19) causing virus is a single stranded RNA virus which has spread across the globe causing human respiratory tract infection. Some of the unconfirmed observations indicate that COVID-19 pandemic is less intense in the countries with BCG vaccine intervention. A reduction in the number of deaths attributed to COVID-19 in countries that have universal BCG vaccination (usually at birth) compared to the countries that never established such policy has been observed. There is some evidence from both animal and human studies that the BCG vaccine has non-specific effects on the immune system. These effects have not been well characterized and their clinical relevance is unknown. Therefore this review discusses probable association of BCG vaccination in protecting against COVID-19 infection.

COVID-19 virus

Corona viruses (CoVs) belong to the subfamily *Orthocoronavirinae* of the *Coronaviridae* family (order *Nidovirales*) classifies into four genera of CoVs: Alphacoronavirus (alphaCoV), Betacoronavirus (betaCoV), Deltacoronavirus (deltaCoV), and Gammacoronavirus (gammaCoV). These viruses represents crown-like spikes on their outer surface due to the presence of spike glycoproteins on the envelope, thus it was named as a corona virus (*coronam* is the Latin term for crown). Corona viruses are minute with a diameter of 65-125 nm. They belong to group of RNA virus and contain a single-stranded RNA (ssRNA) as a nucleic material. There are four common human corona viruses: 229E, NL63, OC43, HKU1 which usually cause mild to moderate symptoms. Apart from them three additional corona viruses which were thought to infect only animals evolved over the time and were eventually transmitted to humans and the world witnessed a severe acute respiratory syndrome outbreak caused by SARS-CoV, 2002 in Guangdong, China (Zhong et al., 2003). Almost after a decade another pathogenic corona virus, known as Middle East respiratory syndrome corona virus caused an endemic in Middle Eastern countries especially in Saudi Arabia (Wang et al. 2013). The novel virus was named as Wuhan corona virus or 2019 novel corona virus (2019-nCov) by the Chinese researchers. The International Committee on Taxonomy of

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Viruses named it as SARS-CoV-2 and the disease as COVID-19 (Lai et al., 2020; WHO, 2020) The A strain was the responsible for corona virus outbreak that originated in Wuhan in China but B strain was responsible for a majority of infections in Wuhan and East Asia but various mutations were observed in different countries.

The International Pharmaceutical Federation Health Advisory issued updated clinical information and treatment guidelines for treatment of COVID-19 infection on 26th march 2020 which mentions that, there is no specific medicine or vaccine for COVID-19 and none of medicines or vaccines have been fully tested for safety and efficacy. At present for the treatment of COVID-19, antiviral therapy is mainly used, along with symptomatic and supportive treatment based on the clinical condition of the patient. Supportive treatments include oxygen therapy, hydration, fever/pain control, and antibiotics if there is associated bacterial co-infection (FIP 2020).

According to the diagnosis and treatment plan recommended by the Chinese health authorities, the antiviral drugs that can be tested for treatment mainly include Interferon-α (INF-α) as aerosol inhalation therapy, lopinavir/ritonavir, ribavirin, chloroquine phosphate, umifenovir and others (Li et al., 2020). World Health Organization (WHO) recommended Remdesivir as most promising candidate based on the broad antiviral spectrum but lopinavir/ritonavir, either alone or in combination with Interferon beta (INF-β), is considered a suitable option (WHO 2020).

Out of the drugs mentioned INF-α which is produced by recombinant DNA technology produces effect similar to interferon (INF) an endogenous substance which is a glycoprotein. All INFs are from the family of cytokine mediators critically involved in alerting the cellular immune system to viral infection of host cells (Samuel, 2001; Scagnolari et al., 2013). Interferon-I (INF-I) is produced in large quantities in response to viral infections and are generally regarded as a key bridging mechanism between innate and adaptive immune responses, exerting both antiviral activity and immunostimulatory functions.

Role of immunomodulation to combat infection

Direct therapy of viral infections is a medical problem as the drugs affecting viral function also affect host cells, producing toxicity. Therefore along with specific antiviral agents, drugs targeting non-specific or specific innate or adaptive immune responses may improve clinical response. As vaccine development is a slow and laborious process, immunotherapy has become a potential suitable alternative for treating viral infections (Hegde et al., 2009).

Immune response is an important self-defense mechanism that protects the host from numerous pathogenic infections. An immune response includes innate and adaptive immunity. Innate immune response occurs immediately when the infectious agents approach the external barrier, whereas the adaptive response causes the formation of immunological memory that allows a quicker and more effective response upon next encounter with the same pathogen (Mak et al., 2014).

During the first critical period of exposure to a new pathogen, innate immune system helps in protection against infection. Innate immune responses are not specific to a particular pathogen in contrast to adaptive immune responses. Innate immune response depends upon a group of proteins and phagocytic cells that recognize few specific features of pathogens and become quickly activated to help to destroy invaders. This immune system depend upon the recognition of particular site at pathogens that are common to many pathogens but are absent in the host. These pathogen-associated molecules also called as, pathogen-associated immunostimulants (PAIs) stimulate two types of innate immune responses-inflammatory responses and phagocytosis. Both of these responses can occur quickly, even if the person is not being previously exposed to the particular pathogen (Takaoka et al., 2006). PAIs act as a very potent chemoattractants for neutrophils, which migrate quickly to the site of such stimulants and engulf the organism that are producing the stimulus. The various classes of PAIs often occur on the pathogen surface in repeating patterns. They are recognized by several types of dedicated receptors in the host that are collectively called *pattern recognition receptors*. These receptors include soluble receptors in the blood (components of the complement system) and membrane-bound receptors on the surface of host cells. The membrane bound receptor on host cells are members of the Toll-like receptor family (TLR). TLR present in macrophage and neutrophil enable them to recognize and engulf pathogens. They act as an alarm system to alert both the innate and adaptive immune systems as soon as infectious organism enters in body (Janeway Jr. et al., 2002).

Immune response to viral infection (Koyam et al., 2008)

The PAIs on the surface of bacteria and parasites that are so important in eliciting innate immune responses are generally not present on the surface of viruses. Viral proteins are constructed by the host cell ribosomes and the membranes of enveloped viruses are composed of host cell lipids. The only unusual molecule associated with viruses is the double-stranded RNA (dsRNA) that is an intermediate in the life cycle of many viruses. Host cells can detect the presence of dsRNA and initiate a program in attempt to eliminate it. The program occurs in two steps:

First the cells degrade the dsRNA into small fragments (about 21-25 nucleotide pairs in length). These fragments

bind to any ssRNA in the host cell with the same sequence as either strand of the dsRNA fragment, leading to the destruction of the ssRNA.

Second the dsRNA induces the host cell to secrete two cytokines- *IFN-α* and *IFN-β.* The binding of the INFs to their cell-surface receptors stimulates specific gene transcription by the Jak/STAT intracellular signaling pathway leading to the activation of a latent ribonuclease, which nonspecifically degrades ssRNA.

It also leads to the activation of a protein kinase that phosphorylates and inactivates the protein synthesis, initiation of Eukaryotic Initiation Factor-2, shutting down most protein synthesis in the embattled host cell. Probably by destroying most of the RNA it contains and transiently halting most protein synthesis, the cell inhibits viral replication without killing itself. In some cases, however, a cell infected with a virus is persuaded by white blood cells to destroy itself to prevent the virus from replicating. In addition INFs enhance the expression of class I major histocompatibility complex proteins, which present viral antigens to cytotoxic T lymphocyte for further destruction.

The local production of IFN-α and IFN-β activates the killing activity of natural killer (NK) cells. NK cells destroy virus-infected cells by inducing the infected cell to kill itself by undergoing apoptosis. Based on the role of IFNs in enhancing immune function therapeutic potential of the IFNs is currently tried in number of virus-associated diseases. This is one of the agents recommended by WHO along with antiviral drug therapy in treatment of COVID-19 infection.

BCG and COVID-19

Bacillus Calmette Guérin (BCG) vaccine is a vaccine primarily used against tuberculosis (TB) (WHO 2018). Few unconfirmed observations indicate that COVID-19 pandemic is less intense in the countries with BCG vaccine intervention. There is also reduction in the number of deaths attributed to COVID-19 in countries that have universal BCG vaccination compared to the countries that never established such policy has been observed (Zwerling et al., 2011). Italy and the United States, two of the countries hit hardest by the pandemic, do not have universal BCG vaccination policies. In contrast, Japan had some of the earlier cases, but the mortality is low despite not having adopted more restrictive social isolation measurements.

Bacilli were isolated from the cow having tubercular mastitis. Vaccine was developed and named after Calmette and Guerin at Pasteur Institute in Lille, France. BCG vaccines were first used in 1921, subsequently rolled out in developed countries and since 1974, have been included in the WHO Expanded Program on Immunization. This isolate was subsequently distributed to several laboratories in the world and a number of strains developed (Oettinger et al., 1999). Currently, five main strains account for more than 90% of the vaccines in use worldwide with each strain possessing different characteristics. The agreed terminology for the strains include the Pasteur 1173 P2, the Danish 1331, the Glaxo 1077 (derived from the Danish strain), the Tokyo 172-1, the Russian BCG-I, and the Moreau RDJ strain (WHO 2004).

Ordinarily, a vaccine provides protection from a particular pathogen, by inducing effector mechanisms directed to that pathogen. However certain live attenuated vaccines like BCG provide protection not only to a specific pathogen, but also against unrelated pathogens, some of which cause acute respiratory tract infections (Aaby et al., 2014; Hollm-Delgado et al., 2014).

BCG in non TB Acid fast bacilli

Even though BCG vaccine was developed to combat TB, but it is observed that the mortality benefit from BCG is not TB-specific but can be effective against some other organisms. Non-tuberculous mycobacteria (NTM) also called atypical mycobacteria can cause infection in lungs, skin and lymphatics. The effects of BCG vaccination on NTM infections especially Buruli ulcer disease was analyzed by Zimmermann et al. (2018) in a systematic review. The analysis revealed that BCG is protective against NTM lymphadenitis in children.

Evidence on the efficacy of BCG to prevent leprosy is well established (Merle et al., 2010), even though there is no guideline issued by WHO which recommends its use as a leprosy preventive vaccine. BCG is already a part of the vaccination policy of most leprosy endemic countries.

BCG in other infections

BCG vaccination has shown to provide partial protection against neonatal sepsis and respiratory tract infection (Hollm-Delgado et al., 2014). Such protection is not limited to neonates and children, BCG vaccinated elderly (age 60-75) individuals have shown to experienced decreased respiratory infections (Wardhana et al., 2011). A large number of data from animal studies provide strong evidence for BCG's ability to protect against a wide range of infections other than TB. The non-specific immune benefits of BCG have been known since the 1970s when BCG was shown to improve immunity against listeria and influenza in murine models (Spencer et al., 1977; Ratzan et al., 1972). The immunomodulatory properties of BCG have been explored in *invitro* experiments for decades in animal model against bacteria (e.g. *Shigella flexneri*), viruses (e.g. vaccinia virus) and protozoa (e.g. malaria) which was reviewed by Freyne et al. (2015).

In a randomized placebo-controlled human study, BCG vaccination was demonstrated to induce epigenetic reprograming in monocytes, providing protection against experimental infection with an attenuated yellow fever virus vaccine strain (Arts et al., 2018). A systematic review by Kandasamy et al. (2016) analyzed 37 studies which measured non-specific immunological effects (NSIE) of BCG vaccination. The limitation of this study was that all research studies analyzed had very heterogeneous designs, which could not be conventionally meta-analyzed, therefore it does not provide uniformity and low strength of reliability. The authors concluded that, few of the studies showed evidence suggestive of NSIE, but no consistent findings were identified to reach to firm conclusion on role of BCG vaccination in providing NSIE in humans following BCG vaccination.

Mechanism of NSIE of BCG vaccination

It is likely that the NSIE of BCG is mediated partly by heterologous effects on adaptive immunity and induced non-specific protection. Netea et al. (2011) were the first to propose the concept of "trained immunity" which is defined as an increased non-specific response to a secondary infection mediated by the innate immune system, either to the same or different microorganisms. This type of immunity is characterized as being independent of T and B cell responses and is mediated by monocytes /macrophages and NK cells. In humans BCG vaccination of adults induces a trained phenotype in circulating monocytes, characterized by an increased capacity to produce proinflammatory cytokines, an effect that has translated to non-specific protection against unrelated pathogens. Trained-immunity inducing agents reprogramme bone marrow hematopietic stem cells and multipotent progenitors through epigenetic and metabolic changes, resulting in a more robust response in differentiated innate immune cells, following encounter with a pathogen (Moorlag et al., 2019).

After BCG vaccination in healthy human volunteers increased capacity of NK cells to secrete proinflammatory cytokines, such as IL-1β and IL-6 after stimulation with M. tuberculosis or unrelated pathogens (*S. aureus, C. albicans*) was observed (Kleinnijenhuis et al., 2014). These observations were performed 3 months after vaccination, which is consistent with the fact that BCG reduces mortality in newborns during the first year of life, as mentioned above.

Correlation of COVID-19 infection with BCG vaccination

Even though the tuberculosis vaccine does not directly protect against COVID-19 it has been thought to boost the immune systems which was suggested by few observations (Jop de, 2020). The analysis of data of various studies was done by Miller et al. (2020) and Hegarty et al. (2020) showing trend of reduced mortality due to COVID-19 in countries with universal BCG vaccination policy. On 11 April 2020, WHO updated its ongoing evidence review of the major scientific databases and clinical trial repositories, for COVID-19 and BCG (WHO 2020c). The review yielded three preprints (manuscripts posted online before peer-review), in which the authors compared the incidence of COVID-19 cases in countries where the BCG vaccine is used with countries where it is not used and observed that countries that routinely used the vaccine in neonates had less reported cases of COVID-19 to date. Such ecological studies are prone to significant bias from many confounding factors, like differences in national demography, disease burden, testing rates for COVID-19 virus infections, and the stage of the pandemic in each country.

The review also yielded two registered protocols for clinical trials, both of which aim to study the effects of BCG vaccination given to health care workers directly involved in the care of patients with COVID-19. These studies are the Phase III BRACE clinical trial, an Australian study sponsored by the Murdoch Children's Research Institute in collaboration with The Royal Children's Hospital, and the Phase III BCG-CORONA trial, a Dutch study sponsored by the University Medical Center Uterecht in collaboration with Radbound University. Both trials are designed to investigate the effect of BCG vaccination in non-infected healthcare workers to determine if BCG vaccine is able to provide innate immunity against COVID-19 in placebo-controlled trials. The patient population of both the studies have slight difference, as the BRACE study has enrolled all healthcare workers whereas the BCG-CORONA trial has enrolled only health personnel who are taking care of patients with a COVID-19 infection. It is therefore probable that the participants of the BCG-CORONA trial will have a higher level of exposure to corona virus, and the study should be able to provide a better indicator of the BCG vaccine's potential protective effects. The end point of both the studies are levels of absenteeism over six months to determine whether there is a significant difference in the level of incidence of COVID-19 between the experimental and placebo cohorts. Although they may provide information on the endpoints outlined in the studies, it may not provide data on a direct correlation on protection from COVID-19, as several COVID-19 positive subjects are asymptomatic, potentially due to inherent immunity (Clinical trial.gov 2020a, Clinical trial.gov 2020b).

Baylor College of Medicine (Clinical trial.gov 2020c) and Ain Shams University (Clinical trial.gov 2020d) are conducting similar placebo controlled clinical trials in population of health workers, and the trials are under Phase IV and Phase III level of development, respectively. The

primary outcome of both of these studies is focused on the incidence of COVID-19 rather than the abseentism. A Phase IV placebo controlled clinical study in the Netherlands sponsored by the Radboud University Medical Centre (GlobalData Healthcare 2020) aims to tests the effectiveness of the BCG vaccine against COVID-19 in an elder patient cohort, where participants are above 60 years of age. The primary outcome is based upon the number of COVID-19 related admissions and aims to evaluate if the administration of the BCG vaccine reduces the rate of hospital admissions in elderly patients. Another study is a Phase II clinical trial in Egypt sponsored by Assiut University (Clinicaltrials.gov.E.2020) which will assesses the severity of disease in COVID-19 positive patients between age group of 12–80 years who have previously been vaccinated with a BCG vaccine versus non-vaccinated patients. Subjects are grouped into two cohorts: those with a positive tuberculin test and those with a negative tuberculin test. This study will be very useful in assessing if the BCG vaccination decreases the impact of the disease as opposed to whether the vaccine offers immunity. However as the participants would have been vaccinated at different time intervals, the degree of immune-protective effects shown by the vaccine may be variable in the patient cohort and may have an effect on the results.

Taken together, the results of all the listed trials could provide essential data in determining whether the BCG vaccine provides a level of immunity or protection against COVID-19. A positive correlation could prove to be additional target in combating COVID-19 infection. The WHO is of opinion that there is evidence from both animal and human studies that the BCG vaccine has non-specific effects on the immune system, but these effects have not been well-characterized and their clinical relevance is unknown (de Bree et al., 2018; WHO 2018). On 12 April, WHO stated in a press briefing that there is currently no data-based evidence that the BCG vaccination provides protection against COVID-19. There are multitudes of confounding variables that prevent to reach to any firm correlations. However, due to lots of unconfirmed studies WHO has not ruled out the fact that the vaccine may exhibit positive effects in protecting COVID-19 infection or reducing its severity. In the absence of evidence, WHO does not recommend BCG vaccination for the prevention of COVID-19.

Conclusion

Analysis of the results of all the listed experimental studies and clinical trials, there seems to be a trend to indicate partial protection or a level of immunity of BCG vaccine in treatment of COVID-19 infection. However it is difficult to translate experimental data to clinical efficacy. It is difficult to reach to any conclusion by meta-analysis due to variation in study design, disease burden and analysis of end points. Probably after completion of multiple studies which are ongoing, it may be possible to reach to some conclusion. However so far WHO has not recommended BCG vaccination as a treatment guideline.

Conflict of interest

Nil

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