Correlation Of BCG Vaccination And COVID-19 Disease Through Measurement Of Immunological Markers IFN-Γ And Anti-BCG Igg In Different Age Groups Running Title: Correlation Between BCG Vaccination And COVID-19 Disease

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KEYWORDS
BCG vaccine, COVID-19, anti-BCG IgG, IFN γ level

ABSTRACT
Introduction Coronavirus disease (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in China in December 2019 and then became a pandemic. BCG vaccination has conferred non-specific (targeted-off) protective effects against unrelated pathogens including respiratory viral infections that have been hypothesized to it could reduce the severity of COVID-19 disease.
Objective: Assess whether BCG vaccination has a role in the prevention of COVID-19.
Methods: A cross-sectional study including 111 healthy people was carried out from October 2021 to December 2022. Specific anti-BGC-IgG and IFN-γ levels were estimated in blood samples after stimulation with BCG vaccine for 2 days. All participants were examined for BCG scar status and COVID-19 confirmation.
Results: There was no significant difference in anti-BCG IgG levels or IFN-γ levels ($p>0.05$) in relation to COVID-19 infection. There was no statistically significant association between BCG scar diameter and COVID-19 infection ($p>0.05$). Furthermore, there was no statistically significant association between the BCG scar diameter and COVID-19 severity ($p>0.05$). There was no significant difference in anti-BCG IgG levels ($p>0.05$) in relation to COVID-19 severity. Likewise, there was no significant difference in between IFN γ levels and COVID-19 severity ($p>0.05$).
Conclusion: There was no correlation between BCG vaccination and infection or severity of COVID-19.

1. Introduction

Despite the principal use of BCG to protect against TB, numerous studies showed its ability to induce non-targeted protection against unrelated infections. A case-control study in Guinea-Bissau indicated BCG can reduce the incidence of neonatal acute lower respiratory tract infections [1]. These non-specific protective effects of BCG were found to enhance resistance against viral infections, such as respiratory syncytial virus, herpes virus, human papillomavirus, and influenza A virus [2].

Non-specific effects from BCG are mediated protection via enhanced innate immune response, activation of heterologous lymphocytes, and enhanced production of cytokines [2]. Since December 2019, an outbreak has emerged in China by a novel severe acute respiratory syndrome coronavirus 2(SARS cov-2) and then became a pandemic worldwide [3].

Although WHO directions do not recommend BCG vaccination due to the absence of evidence of its protection against COVID-19. Initial databases established that longstanding and global BCG vaccination policies in some countries were associated with decreased severity of cases and mortality of COVID-19 compared with others who have not used it [4,5]. The aim of the present study is to determine whether BCG vaccination has a role in the protection or alleviation of COVID-19 severity.

2. Methodology

A total of 111 BCG-vaccinated healthy individuals aged from 1.6 to 60 years old were included in the study, during the period from October 2021 to December 2022. The participants' informed consent was obtained orally. The questionnaire included age, gender, Diabetes Mellitus, allergies, other chronic diseases, TB family history and tuberculin test, education level, occupation, and socioeconomic status.

The classification of COVID-19 severity (mild, moderate, severe) was verified according to WHO criteria. The diagnosis was confirmed by clinical signs and symptoms, PCR, rapid antibody test, and

chest X-ray and CT scan. BCG vaccine status was determined by the presence of a BCG scar on the deltoid area of the left arm. The result was recorded as < 5mm, 5mm,> 5mm. The people without scars were asked about previous BCG vaccinations. The exclusion criteria were those who had active TB or with IGRA positive results, a history of chronic disease, and pregnant women.

Sample collection and culture of whole blood: About 5 ml of heparinized blood via venous puncture was collected from each individual under sterile conditions and distributed equally in labeled lithium heparinized sterile tubes. Within 2 hrs. of collection, 200 µl of heparinized blood were diluted 1:4 in 600 µl of Rpmi-1640 tissue culture medium supplemented with 2 ml M L-glutamine,100IU /ml penicillin, diluted blood was dispensed in 24-well tissue culture plates, each diluted sample was stimulated with 100 µl /well of BCG vaccine Tokyo strain to give final concentration 10^5 cfu of M. bovis BCG. Some of the wells not induced with BCG vaccine were left untreated as negative control. Plates were incubated in a humidified atmosphere (37˚c,5% CO2) for 2 days. After that culture supernatants were collected as aliquots and kept at -40c˚ until analysis. The kit of IFN-γ detection limits is 15.625-1000pg /ml while the kit sensitivity is > 9.375 pg/ml, anti-BCG -IgG titer is determined relative to anti-BCG IgG Calibrators which were performed to specifications: 3 U/ml,10U/ml and 30U/ml and Control value: (8-16) U/ml.

The statistical analysis was carried out with SSPS (Statistical Package for the Social Sciences) version 26. Categorical data were formulated as frequencies and percentages. The chi-square test and Fisher's exact test (where appropriate) were used to test the significance of the association between the categorical variables.

Numeric variables were described as mean, standard deviation, and ranges. Median was used for data that is not normally distributed. For data that are normally distributed, A paired t-test was performed to compare the means before and after stimulation. In-depended samples t-test was used to compare means for unrelated groups. For data that are not normally distributed, a sign test was used to compare the medians before and after stimulation. Mann-Whitney U test was the test of significance to compare medians of unrelated groups.

Pearson Correlation was used to describe the correlation of two continuous variables. The statistical significance is known as a P-value less than 0.05.:

3. Results and discussion

Out of 111 participants in this study, 47 (43.2%) of them had a confirmed COVID-19 infection. Furthermore, only 39.6% (no=44) of the individuals had received COVID-19 vaccine. The COVID-19 infection test results in addition to their vaccination status are shown in Table 1. BCG vaccine scar, scar was absent in 7 (6.3%) of the participants, and most of them had more than 5mm in diameter (64%). Table 2 shows the diameter of BCG scars among subjects. The COVID-19 infection test results in addition to their vaccination status are shown in Table 3.

Among the 47 infected participants, 32 (68.1%) of them had a mild COVID-19 infection, 13 (27.7%) had a moderate infection, and two had a severe infection (4.3%). Additionally, five (4.5%) of the infected participants were hypertensive and six (5.4%) had a history of seasonal allergy as demonstrated in Table 4.

The associations between Interferon-gamma, anti-BCG IgG, and scar diameter with the COVID-19 infection are demonstrated in Table 5. There was no significant difference in Anti-BCG IgG levels ($P > 0.05$) in relation to COVID-19 infection. Similarly, there was no statistically significant difference in regard to interferon-gamma levels and COVID-19 infection status, ($P > 0.05$). Furthermore, there was no statistically significant association between scar diameter and COVID-19 infection, ($P > 0.05$).

The associations between Interferon-gamma, anti-BCG IgG, and scar diameter with the severity of

COVID-19 infection are demonstrated in Table 6. There was no significant difference in Anti-BCG IgG levels (P > 0.05) in relation to COVID-19 severity. Likewise, there was no statistically significant difference between interferon-gamma levels and COVID-19 severity (P > 0.05). Furthermore, there was no statistically significant association between the BCG scar diameter and COVID-19 severity (P > 0.05).

Discussion:

There was no impact on the BCG scar diameter or levels of INF-γ and BCG-specific IgG antibodies on the severity of COVID-19 infection (p< 0.05). A study by Caliskaner et al.,2022, reported there was no relation found of BCG scar (which was confirmed by the presence and the number of scars in each participant) when compared with and without a history of COVID-19 hospitalization and intense-care admission [6]. Also, from the results of our study, we can conclude there was no impact of BCG vaccination on the prevention of infection with Covid-19. These results agree with a study by Khanum I, et al.,2021. [7]. BCG vaccination in elderly adults who were infected with COVID-19 showed a protective effect against COVID-19 severity through improvement in vitro cytokine production and antibody titer when compared with unvaccinated ones [8]. In a randomized trial conducted on healthcare workers after 6 months of BCG vaccination, it did not lower the risk of COVID-19 [9].

In contrast to these findings, several studies performed in Japan, Indonesia, South Africa, and Greece indicated a protective role of BCG vaccination on respiratory tract infection incidence in adults [10-14]. Also, another study in the United Arab Emirates found that BCG revaccination can prevent COVID-19 infection in high-risk healthcare workers who had no infection with COVID-19 in comparison with 8.6% of the unvaccinated population [15]. By using gene expression of monocytes after administration of BCG vaccine in individuals was found that BCG vaccination can enhance immunity response against SARS-Cov. 2 but not prevent its infection [16].

In a study on a cohort of BCG-vaccinated individuals, it was found there were low hospitalization requirements for these patients [17]. A study by (ChauhanA.et al Oct-Dec 2021), conducted on health workers who were exposed to low and high risk of COVID-19 infections in hospitals correlated BGC developed in individual arms associated with a lower number of COVID-19 patients but did not support its role in the development of the disease [18]. A study on a large cohort of childhood BCG-vaccinated and unvaccinated individuals did not find differences in COVID-19 incidence [19].

4. Conclusion and future scope

There was no impact of BCG vaccination immune response and BCG scar status and IFN-γ and anti–BCG IgG levels on infection of COVID-19 or its severity.

Acknowledgment: The authors have profound thanks and appreciation to Dr.Hnadi Abd Al-Qadir for her assistance in proofreading.

Conflict of interest: The authors have no conflict of interest.

Funding: Research is self-funded

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