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EVALUATION OF THE ANTIBODY LEVELS AGAINST BCG AND POLYSACCHARIDES MENINGOCOCCAL VACCINES WHEN COMBINED, CO-ADMINISTRATED OR ADMINISTRATED AT DIFFERENT INTERVALS IN MURINE MODEL

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ABSTRACT

Bacillus Calmette-Guerin (BCG) and polysaccharide meningococcal (pMen) vaccines are required to travel to some countries, medical staff to deal with disease carriers, and for special cases as well. However, there were not enough studies on the immunogenic intervention between them when given together or in combination. Hence, we tried to assess the antibody-mediated immune response in groups of mice when different BCG vaccines and pMen were combined or given together, either concurrently or with a 14-day break between them. The antibody titer was measured in each group after 21 and 42 days from the first injection. Our results showed that it was safe and effective to give these two vaccines combined or simultaneously. Moreover, they exhibited a potent synergistic effect even if there was a 14 days gap between their administration. However, more research is needed to investigate the immunological intervention's cellular-mediated response.

Keywords: BCG, meningococcal, vaccine, combined, co-administration

INTRODUCTION

Tuberculosis (TB) is a disease caused by Mycobacterium tuberculosis (Mtb) which is considered as a major health problem in developing countries. This disease affects the lungs, bones, joints, and kidneys as well, and can lead to meningitis. Even in industrialized countries, the incidence of TB has grown mainly due to outbreaks among vulnerable groups **((NHS), 2019; (WHO), 2020a)**. The gravity of TB is further complicated by the emergence of mycobacterial antibiotic resistance. Since the 1920s, the Bacillus Calmette-Guerin (BCG) vaccine has been the solely available vaccine to protect against tuberculosis, and about three billion individuals have been vaccinated **((WHO), 2020a; Agger & Andersen, 2002; Wang *et al*., 2009)**. BCG vaccine is not only used for TB protection but also gives some protection against other infections **(Aaby, Kollmann, & Benn, 2014)**, such as meningitis **((WHO), 2020a)**, influenza virus **(Leentjens *et al*., 2015)**, the secondary infection caused by *Candida albicans* **(Van't Wout, Poell, & Van Furth, 1992)**, and *Schistosoma mansoni* **(Tribouley, Tribouley-Duret, & Appriou, 1978)**. Moreover, BCG vaccination may improve the functions of antigen-presenting cells, supporting the increase of antibody titers against influenza H1N1 2009 strain when administrated 14 days later after BCG vaccination **(Leentjens *et al*., 2015)**, the enhancement of the heterologous responses to vaccination with poliovirus **(Libraty *et al*., 2014)**, *Pneumococcus*, *Haemophilus influenza* type b, tetanus toxoid **(Ritz, Mui, Balloch, & Curtis, 2013)** and hepatitis B vaccine **(Ota et al., 2002)** in BCG vaccinated infants. BCG vaccine has been also successfully incorporated into cancer treatment **(Saad, Hincal, & Kaymakamzade, 2017; Uyl-de Groot *et al*., 2005)**. Some researchers have found a link between BCG immunization and protection against COVID-19 as well. **(Escobar, Molina-Cruz, & Barillas-Mury, 2020; Madan et al., 2020; Malik et al., 2020; Mohamed Hussein et al., 2020; Osama El-Gendy *et al*., 2020)**. Hence, researchers are paying closer attention to the interactions between the BCG vaccine and other pharmaceuticals and biological products.

Meningococcal disease is a dangerous bacterial infection that causes meningitis and septicemia. If left untreated, it can lead to mortality in 50% of patients and lifelong damage (e.g., deafness) in 10% to 20% of survivors **((WHO), 2020b)**. It is caused by the bacteria Neisseria meningitidis where there are twelve serogroups of them; six serogroups (A, B, C, W, X, and Y) were shown to be responsible for epidemics **(Chang, Tzeng, & Stephens, 2012; Stephens, Greenwood, & Brandtzaeg, 2007)**. Meningococcal vaccinations have been available for over 40 years. Vaccines can be monovalent (just one serotype) or multivalent (many serotypes) and they provide varying levels of protection **((WHO), 2020b)**. A multivalent meningococcal vaccine (Men) is either mixture of polysaccharides (the sugar long chains that come from the surface capsule of the bacteria) or a mixture of polysaccharides bound to a carrier protein **(Reyes et al., 2014; Tan, Carlone, & Borrow, 2010)**. Although the conjugated meningococcal vaccines confer long-lasting immunity (≥ 5 years) and are commonly preferable to be used in routine immunization, the polysaccharide vaccines offer about 3-years of immunity and are widely used in response to outbreaks **((CDC), 2011)**. The polysaccharide vaccines are mainly used in Africa due to their lower cost than the other types and among pilgrims visiting Mecca for Hajj or Umrah **(Wilder-Smith, 2007)**.

While simultaneous administration helps increasing the immunization rates, this approach must be supported by safety and immunogenicity data. The main concern is immunological interaction, which refers to the relative enhancement or suppression of immune responses when vaccines are given together rather than individually. This information is useful in making policy decisions about whether or not to include a vaccine in the designed immunization schedule. Both the BCG and meningococcal vaccinations are tested in different ways to see how they immunologically interact with other vaccines. **(Abbas, Rashed, El-Gebaly, AbdelAllah, & Gaber, 2022; Alderfer *et al*., 2019; Ali, Abdeltawab, Hady, & Amin, 2017; Badahdah, Tashani, Khatami, Booy, & Rashid, 2018; Gasparini *et al*., 2016; Leentjens *et al*., 2015; Libraty *et al*., 2014; Ota *et al*., 2002; Ritz, Hanekom, Robins-Browne, Britton, & Curtis, 2008; Ritz *et al*., 2013)**. However, there was no enough evidence to evaluate the immune interaction between BCG and polysaccharides meningococcal vaccination.

The objective of our study was to evaluate the combination, or co-administration of Egyptian registered BCG and polysaccharides meningococcal vaccines (pMen), we used two different commercial BCG vaccines against pMen and evaluated their antibody response in mice. We assessed several experimental designs where each vaccine was administrated in combination, co-administration at the same time, or with a 14-day gap interval in comparison with each vaccine alone.

MATERIALS AND METHODS

Balb/C mice

Mice (6 weeks, 17-22 g) from VACSERA's animal facility (Helwan, Egypt) were kept in a controlled environment and supplied with regular meals and free access to water. One week before the study began, mice were divided into different groups for environment customization. The experiments were carried out following the international guideline (Council, 2011).

Vaccines

Mencevax® (MVX) is the free polysaccharide (PS) vaccine that contains 100ug/ml of free PS (A, C, W135, and Y serogroups) per human dose (0.5 ml).
Two different BCG® vaccines were used in this study, (BCGs) (Serum Institute of India, India) of live attenuated Russian BCG-I sub-strain and BCG® vaccine (BCGg) (Green signal, India) live attenuated BCG-Danish 1331 strain. Both vaccines contain about 5×10^6 CFU of BCG per human dose (0.1ml).

Preparation of combined vaccines

The combined vaccine of either BCGs or BCGg with MVX (BM1 and BM2 respectively) was prepared by reconstituting the lyophilized MVX vials of 10 doses with 5ml of sterile saline till complete dissolving, then the reconstituted solution was used to dissolve the dry powder of BCG 10 doses vial. The final solution contained 5×10^6 CFU of BCG plus 100ug/ml of free Men PS serogroups per 0.5 ml.

Study design of immunization

The mice were arranged in groups (n = 12 mice /group) as follows:
Group 1/2 (BCG1)/(BCG2): with 0.1 ml of (BCGs)/(BCGg) intra-muscularly (IM) containing 5×10^6 CFU of BCG at day 0;
Group 3 (Mvx): with 0.5 ml of MVX vaccine subcutaneously (SC) at day 0 and day 21;
Group 4/5 (BM1)/ (BM2): with 0.5 ml of BM1/BM2 SC at day 0, then with 0.5 ml of MVX vaccine SC on day 21;
Group 6/7 (BCG1/Mvx)/(BCG2/Mvx): with 0.1 ml of (BCGs)/(BCGg) IM at day 0, co-administrated with 0.5 ml of MVX vaccine SC at day 0 and day 21;
Group 8/9 (BCG1/Mvx-14)/(BCG2/Mvx-14): with 0.1 ml of (BCGs)/(BCGg) IM at day 0, and with 0.5 ml of MVX vaccine SC at day 14 and day 35;
Group 10/11 (Mvx/BCG1-14)/ (Mvx/BCG2-14): with 0.5 ml of MVX vaccine SC at day 0 and day 21, and with 0.1 ml of (BCGs)/(BCGg) IM at day 14;
Group 12 (NC): with 0.5 ml saline as a negative control.
Mice's blood was collected after 21 and 42 days of the first injection per1 each vaccine. Sera were separated by centrifugation at 4000 rpm for 10 minutes. The sera were stored at -20°C until testing. The immunization and blood collection schedules were illustrated in Figure 1.

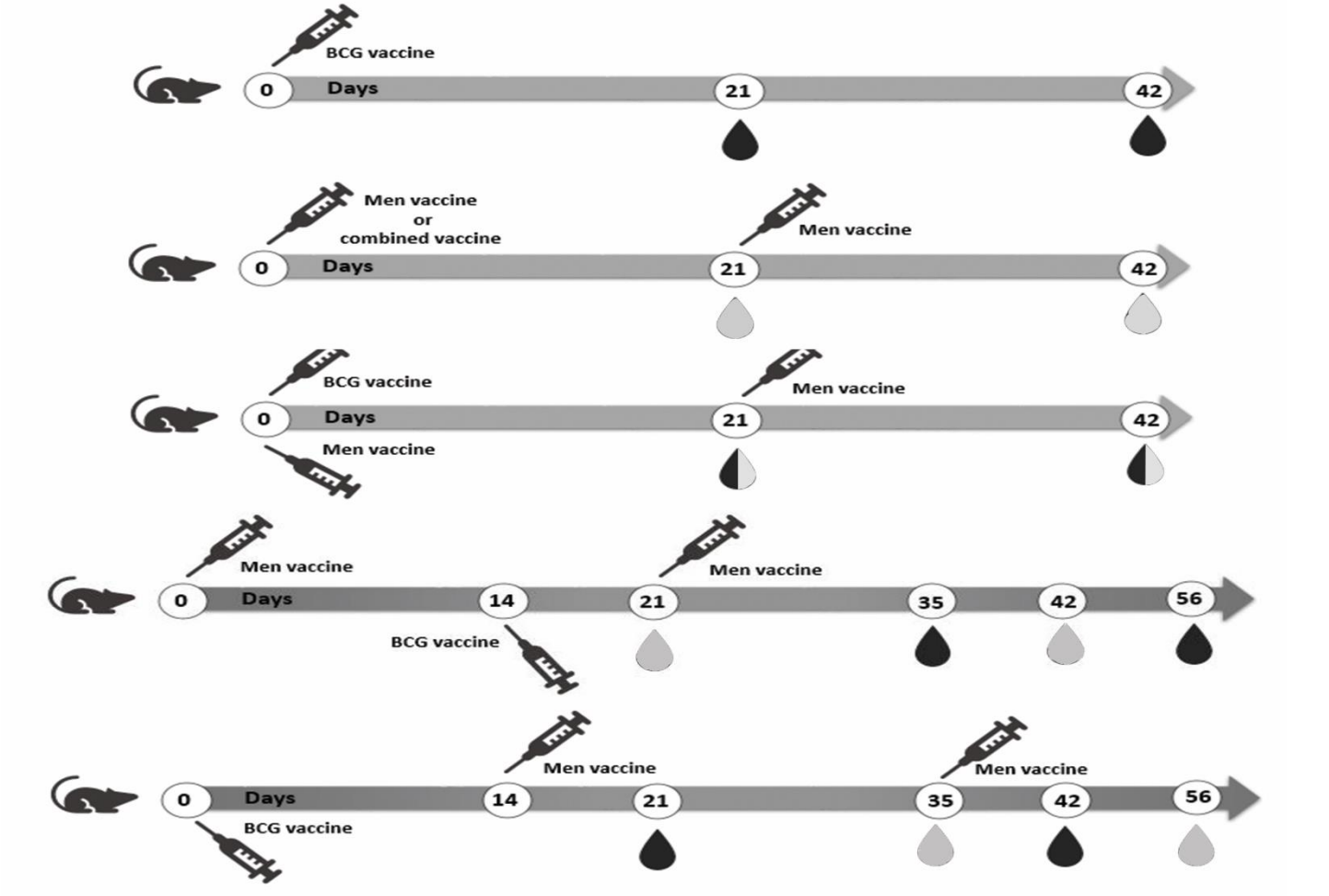

Figure 1 Study design of the mice groups immunization with BCG and Meningococcal (Men) vaccines.
The syringe indicates the immunization time point, while the drop indicates the time point of collecting serum from the mice group (the black drop indicates serum collection for anti-BCG, the grey drop indicates serum collection for anti-Men, and the drop of mixed color indicates serum collection for anti-BCG and anti-Men).

Safety measurement

Each mouse per group was monitored for at least 7 days after injection and the toxicity was assessed by survival rate. Besides, local inflammation symptoms such as redness, swelling at the injection sites, or loss of hair were monitored for each mouse.

Detection of specific antibody responses by ELISA

ELISA was used to determine IgG levels against BCG and Men serotypes (A, C, W135, and Y) in collected serum. ELISA 96-well plates (NUNC, USA) were coated with 10 ug/well of either BCG vaccine or PS of Men serogroups (National Institute of Biological Standers and control (NIBSC), UK) diluted in a carbonate-bicarbonate buffer, pH 9.8, and overnight incubated at 4ºC. Then, plates were washed thrice using phosphate buffer saline (PBS) with 0.05% tween 20. The plates were blocked by bovine serum albumin (SERVA, Germany) 1.5% in PBS, incubated at 37ºC for 2 hours, washed thrice, and 100 ul/well of diluted sera (1/5 for BCG antibodies and 1/50 for Men antibodies) were added at each well. The plates were incubated for 2 hours at 37ºC and washed afterward. The conjugate of anti-mouse IgG peroxidase-conjugate (Sigma, USA) was added (1/4000 and 1/10000) to BCG plates and Men plates respectively. The plates were incubated at 37ºC for 1 hour and then washed again. The substrate TMP (3,3'5,5'-

Tetramethylbenzidine) (Sigma, USA) was added and incubated at 25±3ºC in a dark place for 15-30 minutes. The reaction was stopped with 1 N sulfuric acid (50 ul per well) and optical density was measured at 450/630 nm by an ELISA reader, the analysis was repeated 3 times and the mean of absorbance was computed for the results.

Statistical analysis

The results were represented as the average absorbance ± standard deviation (SD). The results were analyzed using SigmaPlot 12.5 (Systat Software, USA). We used two-way ANOVA followed by the Holm-Sidak method for comparison among different groups. The P values < 0.05 were deemed significant.

RESULTS

Safety assay

All the vaccines incorporated in our study are registered vaccines in several countries. Therefore, all the vaccines have been proven to be effective and safe. However, we observed the mice after each administration to detect any signs of illness or mortality. There was neither mortality nor signs of ill health in the injected mice.

Evaluation of cross-reactive antibody response against BCG and Men serogroups

First, the BCG-antibodies (BCG-Ab) were assessed in the immunized NC and Mvx, group; the antibodies were significantly lower than in BCG groups (P<0.001). Also, the BCG-Ab titers in NC and Mvx groups were equivalent with no significant difference. The same results were obtained when we assessed the antibodies against Men serogroups A (Anti-MenA), C (Anti-MenC), W135 (Anti-MenW), and Y (Anti-MenY) in the BCG and NC groups. Their values were significantly lower than all the other Men groups (P<0.001), and their Absorbance values in NC and BCG groups were similar. Consequently, none of the results proved any cross-reactivity between BCG and Men serogroups.

Measurement of antibody levels against BCG in groups of the combined vaccines

Groups of mice were immunized with pMen and BCG combined vaccines. The sera at 21 and 42 days were collected. Generally, BCG-Ab titers declined from 21 to 42 days in all groups. The BCG-Ab titers in combined vaccinated groups were non-inferior to the titers in BCG vaccinated groups at 21 days from the 1st vaccination dose. On the 42nd day, the titer of BM1 was higher than the BCG1 group (P= 0.02); while the BCG-Ab titer in BCG2 was significantly higher than BM2 (P <0.001) as shown in figure 2A.

Measurement of antibody levels against BCG when co-administered with pMen

We assessed the BCG-Ab titers in the groups vaccinated with pMen and different BCG vaccines at the same time. After 21 days from 1st injection, the titers of BCG-Ab were significantly higher in the BCG1 and BCG2 groups than in BCG1/Mvx and BCG2/Mvx (P=0.004 and 0.003 respectively). While the superiority of the BCG-Ab titer in BCG2 became insignificant compared with BCG2/Mvx after 42 days (P=0.437). On the other hand, the titer in BCG1/Mvx became significantly higher than its titer in BCG1 (P=0.001). Again, the BCG-Ab titers in BCG1/Mvx and BCG2/Mvx decreased from 21 to 42 days as shown in figure 2B.

Evaluation of antibody levels against BCG when administered with pMen at different intervals

We assessed the BCG-Ab titers in immunized groups of mice with pMen and different BCG vaccines with 14 days apart. The BCG-Ab titers in BCG1/Mvx-14 and Mvx/BCG1-14 vaccinated groups were significantly higher than BCG1 after 21 and 42 days of BCGs injection (P<0.001). Moreover, the titer in BCG1/Mvx-14 was notably higher than Mvx/BCG1-14 (P<0.001) at 21 days; however, the opposite was observed after 42 days (Mvx/BCG1-14> BCG1/Mvx-14, P<0.001). On the other hand, the titer of BCG-Ab was significantly higher in Mvx/BCG2-14 than BCG2 at 21 and 42 days; while the titer was significantly increased in BCG2 than BCG2/Mvx-14 (P<0.001) at 21 days then notably decreased after 42 days (BCG2/Mvx-14>BCG2, P<0.001). Finally, Mvx/BCG2-14 produced more BCG antibodies than BCG2/Mvx-14 at 21 days (P<0.001), then the situation is reversed after 42 days (P=0.01). Noteworthy, the titers after 42 days were greater than after 21 days in BCG1/Mvx-14, Mvx/BCG1-14, and BCG2/Mvx-14. The results were presented in figure 2C.

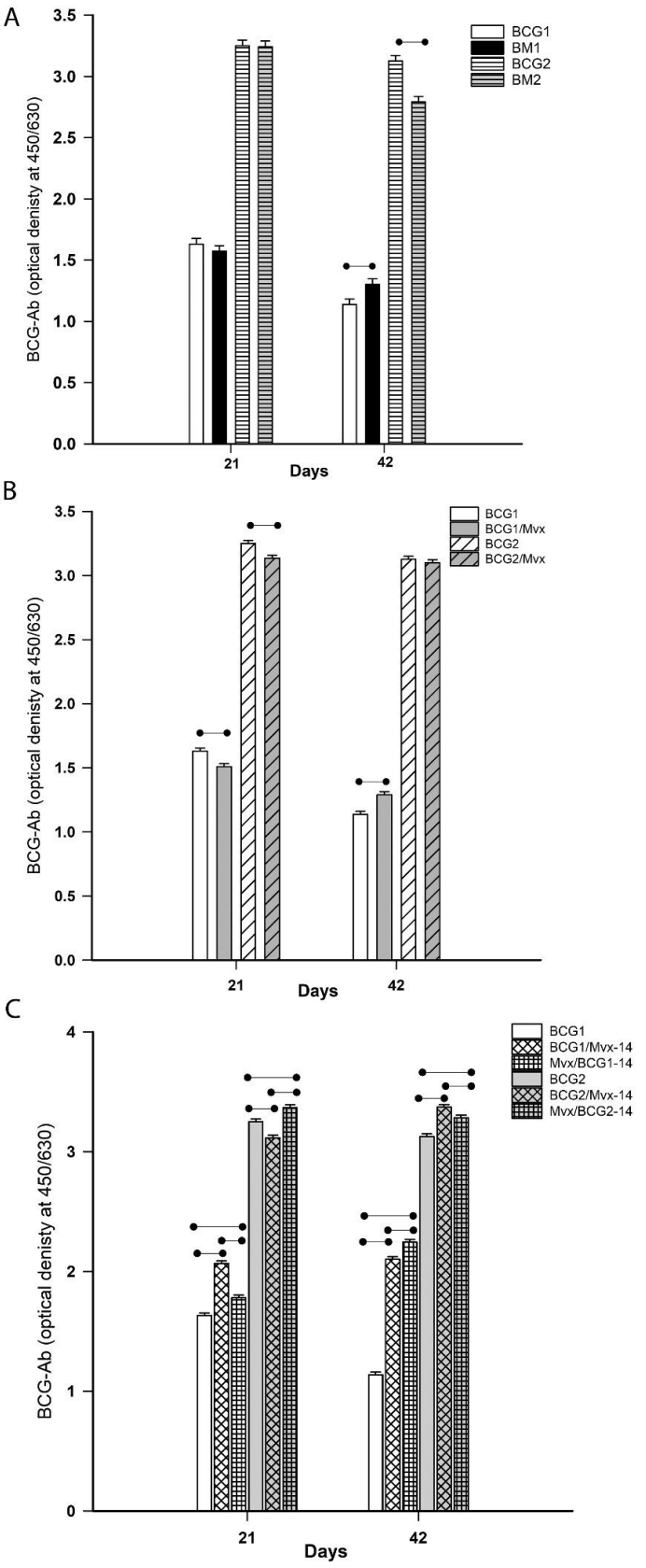

Figure 2 Evaluation of antibody levels against BCG when combined, co-administered, or, sequentially administrated with polysaccharide meningococcal vaccine. Balb /C mice (n=12/group) were injected with different BCG vaccines (BCG1) from Serum institute of India and (BCG2) from Green signal, India, or with polysaccharide meningitis vaccine Mencevax (Mvx). The vaccines were administrated either at once (BCG1/Mvx, BCG2/Mvx) or with 14 days interval between each vaccine [BCG first followed by Mvx vaccine (BCG1/Mvx-14, BCG2/Mvx-14) or Mvx first dose followed by BCG (Mvx/BCG1-14, Mvx/BCG2-14)]. While the combined vaccines of BCG and Mvx (BM1 and BM2 respectively) were injected on day 0 followed by a second dose of the Mvx vaccine at 21st day.

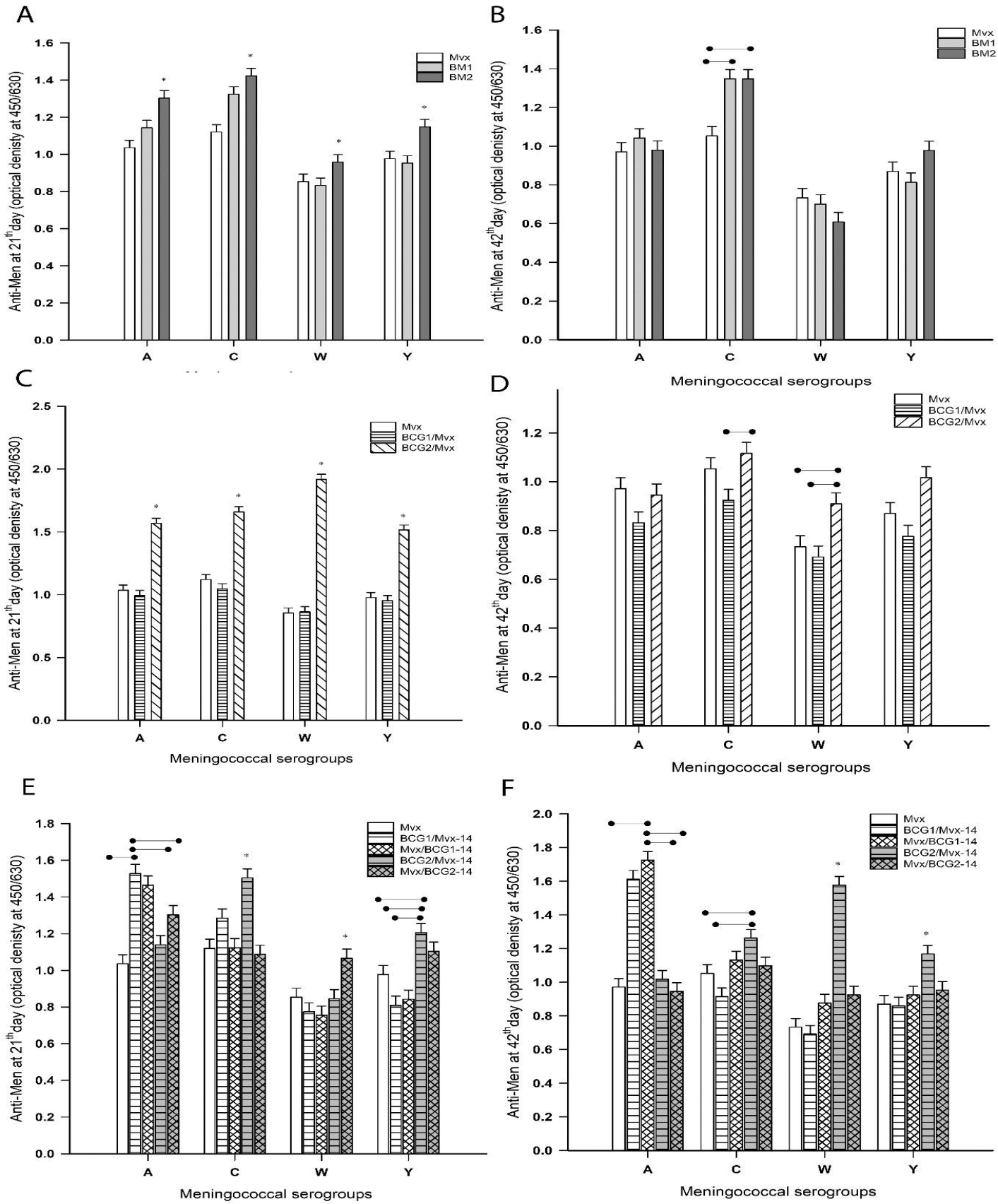

Figure 3 Evaluation of antibody levels against meningococcal serogroups when combined, co-administered, or sequentially administrated with different BCG vaccines. Balb /C mice (n=12/group) were injected subcutaneously with Mencevax (Mvx) alone or combined with of BCG vaccine (BCG1) from Serum institute of India or (BCG2) from green signal, India (BM1, BM2 respectively). The combined vaccines were injected on day 0 followed by a second dose of Mvx vaccine at 21th day. The BCG and Mvx vaccine were also co-administrated either at once (BCG1/Mvx, BCG2/Mvx) or with 14 days interval between each vaccine [BCG1 first followed by Men vaccine (BCG1/Mvx-14, BCG2/Mvx-14) or Men first dose followed by BCG1 (Mvx/BCG1-14, Mvx/BCG2-14)]. Sera from each group were collected after 21 and 42 days from the 1st injection. The antibody levels for ACWY meningococcal serogroups were measured by ELIZA. Each bar represents the absorbance values mean of the optical density at (450/630) ± SD from three measurements. A) combined vaccines BCG with Mvx after 21 days from 1st injection, B) combined vaccines BCG with Mvx after 42 days from 1st injection, C) Mvx with BCG after 21 days from Mvx first dose, D) Mvx with BCG after 42 days from Mvx first dose, E) BCG[redacted]vx administrated 14-days apart after 21 days from Mvx first dose. F) vaccine of BCG with Mvx administrated 14-days apart after 42 days from Mvx first dose. T[redacted]mbol represents there is a significant difference (P<0.05) between the connected groups, while (*) represents that the marked group is significantly higher th[redacted]er groups in the same category (P<0.05).

Sera from each group were collected after 21 and 42 days from the day of BCG vaccination. The antibody levels were measured by ELIZA. Each bar represents the absorbance values mean of the optical density at (450/630) ± SD from three measurements. A) combined vaccine of BCG with Mvx, B) BCG [redacted]istrated with Mvx, C) BCG with Mvx administrated 14-days apart. T[redacted]ymbol represents there is a significant difference (P<0.05) between the c[redacted] groups.

Measurement of antibody levels against meningococcal serogroups for pMen groups of the combined vaccine

We assessed the Men serogroups antibodies in the groups immunized with BCG and MVX combined vaccine. The mean antibodies against Men serogroups became significantly higher in BM2 than Mvx after 21 days in serogroups A, C, and Y. Also, the mean of anti-Men serogroups was higher in BM1 than in Mvx especially serogroups A, and C. Finally, the BM2 produced higher anti-Men than BM1 group at 21st day which was observed notably in A and Y serogroups. While, these increased in the anti-Men serogroups in the combined vaccine groups became insignificant higher than Mvx after 42 days especially serogroups A, W, and Y. Also, the difference in the serogroups anti-Men titer between BM2 and BM1 was insignificant. The results are illustrated in figures 3A-B.

Measurement of antibody levels against meningococcal serogroups for pMen groups when co-administrated with different BCG vaccines

The serogroup anti-Men titers in BCG1/Mvs were non-inferior to their relative titer in the Mvx group whether after 21 or 42 days from the 1st injection. Moreover, the anti-Men titer of all serogroups in BCG2/Mvx was remarkably higher than their relative titer in Mvx group at 21th day (P<0.001). This observation continued even after 42 days especially in serogroups W (P=0.021). Finally, the antibody titers of all serogroups in BCG2/Mvx were significantly higher than their titer in BCG1/Mvx after 21 and 42 days. The results are illustrated in Figure 3C-D.

Measurement of antibody levels against meningococcal serogroups for pMen when administered with BCG vaccine at different intervals

We assessed the antibodies against meningococcal serogroups in the groups immunized with MVX and different BCG vaccines with 14 days between the first injection of each vaccine. In general. Most of the groups produced higher responses compared to Mvx group regardless of the order of the vaccination. The difference in response was insignificant after 21 days between BCG1/Mvx-14 and Mvx/BCG1-14 in all serogroups. This situation was continued after 42days except for serogroup C where the response of Mvx/BCG1-14 was significantly higher than BCG1/Mvx-14 (P=0.04). On the other hand, the difference in the response between the BCG1/Mvx-14 and Mvx/BCG1-14 was insignificant in serogroup A and significant in serogroup W. The responses on BCG2/Mvx-14 were higher than Mvx/BCG2-14 in serogroups C and Y. The results were shown in figure 3E-F.

DISCUSSION

Meningococcal and BCG vaccination are among the national immunization program of many countries for young children (<5 years). Men vaccines are mandatory for all adult pilgrims and occasionally for military officers especially polysaccharide meningococcal vaccine due to its affordable price. Also, BCG vaccination is recommended for adults travelling to the highly exposed areas. While, many clinical studies recommend revaccinated with BCG vaccines whether to reduce the latent Mycobacterium tuberculosis Infection, decrease the SARS-CoV-2 infection sickness as a potential adjuvant, or to mitigate the effects of concurrent respiratory infections **(Gonzalez-Perez, Sanchez-Tarjuelo, Shor, Nistal-Villan, & Ochando, 2021; Madan *et al*., 2020; Suliman *et al*., 2016)**. Our study investigated the immunological interaction of BCG vaccine and pMen (ACWY) vaccine on their antibody levels whether combined in one injection, co-administration at the same time by different syringe in different injection site or administrated with 14 days apart at different order. BCG vaccine is usually intradermally administrated; however, intramuscular route has proven to be well-tolerated and to provoke effective, long-lasting immunity **(Meyer *et al*., 2013; Smrkovski, 1981)**. Therefore, The BCG vaccines were administrated IM in mice. On the other hand, the combined vaccines of BCG and Men vaccine were administrated subcutaneously to deliver 0.5ml as a human dose. First, we assessed the safety profile for the vaccinated mice groups. All the observed mice in each group did not develop any signs of illness or mortality. Even, the BCG vaccination local complications, such as abscess formation or adenitis were not detected in any mouse in the BCG vaccinated groups whether injected IM or SC in the combined vaccinated groups.

pMen vaccines are said to elicit a predominantly antibody-mediated immune response as Thymus-independent antigen **(Cruse & Lewis, 1999; World Health, 2020)**. While BCG depends mainly on cellular immunity as the primary defense. However, many investigations highlighted the importance of the humoral antibodies in the prevention of TB infection by modulating immunity using Fc-receptor mediated phagocytosis **(Glatman-Freedman, 2003; Jacobs, Mongkolsapaya, Screaton, McShane, & Wilkinson, 2016; Lang & Glatman-Freedman, 2006)**. Therefore, the potential of those vaccines to elicit an antibody response in different situations was tested in groups of mice at 21 and 42 days after each vaccine was injected. Generally, the antibodies in all groups were significantly decreased from 21th day to 42th. In the beginning, we checked whether BCG and meningococcal vaccines can evoke cross-reactive antibodies towards each other. We tested the BCG-specific antibodies in mice immunized with Mvx only and vice-versa. The results showed that the antibody titer was equal to the titers in the negative control group. Accordingly, these results prove that neither BCG nor pMen vaccine elicited cross-reactive antibodies for each other. A similar result was proven that vaccination with BCG does not have cross-reactive antibodies against SARS-CoV-2 but does not nullify a possible role in cell immunity **(Kandeil *et al*., 2020)**.

We evaluated the BCG antibody levels in BCG immunized mice groups. Noteworthy, live attenuated BCG-Danish 1331 strain elicited higher antibodies than Russian BCG-I sub-strain live attenuated vaccine either alone or associated with pMen vaccine. This result solidified the study that different BCG vaccine strains have different effects on the immune response and protection against tuberculosis **(Ritz *et al*., 2008)**.

Meanwhile, the combined vaccines had a mixed effect on the BCG antibody levels. The mean titer in BM1 was insignificantly higher than BCG1; however, the opposite finding was observed in the other combined vaccine, where the mean titer of anti-BCG in BCG2 was notably higher than BM2. Of note, the titer of BM2 was non-inferior to BCG2 after 21 days of 1st dose administration. Co-administrated vaccinated groups (BCG1/Mvx and BCG2/Mvx) gave comparable mean anti-BCG titers to their relevant individual groups (BCG1 and BCG2 respectively). Noteworthy, the mean of BCG antibodies in combined vaccinated groups (BM1 and BM2) was equivalent to co-administrated groups (BCG1/Mvx and BCG2/Mvx respectively). The combination effect of BCG and different Men vaccines was also assessed on the antibody titers of meningococcal serogroups. Generally, it was found that the combinations of BCG with Mvx have increased the Men-serogroups antibodies especially after 21 days from the first dose, then the effect became less observable on the 42nd day. Of note, the BM2 combined vaccine had a greater positive impact on Men-antibodies compared to BM1. Meanwhile, the mean of serogroups anti-Men titers in BCG1/Mvx was equivalent with Mvx, even significantly higher in BCG2/Mvx than in Mvx. Notably, the mean of serogroup antibodies in BCG2/Mvx was significantly higher than BCG1/Mvx. Moreover, the combined vaccines elicited significantly more antibodies against meningococcal serogroups than simultaneously administrated. These findings support that concomitant administration of BCG and pMen vaccine, whether combined in one syringe or administrated with different syringes in different sites, as they showed no serious negative immunogenic intervention but even may have a synergic effect on each other. These results comply with the field trial report in Sudan that confirmed the safety and effectiveness to give BCG and bivalent pMen (A and C) vaccine simultaneously **(Omer, el dawla, Nicolas, Roumiantzeff, & Lapeyssonie, 1986)**.

Meanwhile, the BCG antibodies increased significantly compared to BCG groups when BCG and pMen vaccine were administrated 14 apart. This impact did not change whether the BCG vaccine was administrated first followed by Men vaccines or vice versa. Resemblant findings were observed for mean Men-antibodies titers, as they were notably higher in the groups BCG/Mvx-14 and Mvx/BCG-14 compared to the Mvx group. Noteworthy, the mean Men- serogroups antibodies of BCG1/Mvx-14 were significantly lower than Mvx/BCG1-14. On the contrary, the Men antibody mean titer was significantly higher in BCG2/Mvx-14 compared to Mvx/BCG2-14. Finally, the strain of the BCG vaccine has no significant impact on the Men antibodies when the MVX was administrated first followed by BCG vaccination. Meanwhile, the Men antibody mean titer was significantly higher in BCG2/Mvx-14 than BCG1/Mvx-14. This observation indicated that the higher the potency of BCG vaccine, the higher the synergetic effect on the pMen immune response when administrated first followed by pMen vaccination.

CONCLUSION

Based on our results, polysaccharides Men vaccine whether combined with different BCG vaccine or co-administrated instantly exhibited some synergistic effects on each other antibody responses. Moreover, the immune response was much better when the two vaccines were separated with 14 days regardless of the order of their administration. However, because our research focused on protective antibody-mediated immunity, the functions of alternative immune pathways should be investigated further.

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