Original Article

Hematological Parameters Changes in Albino Rats Vaccinated with Bacilli Calmette-Guerin

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Abstract

Scientific background: The Bacillus Calmette-Guérin vaccine (BCG) against tuberculosis (TB) has beneficial protection against TB. This study investigated changes in hematological parameters in albino rats following BCG vaccination. **Method:** An experimental study on the freeze-dried BCG strain (Danish 1331) was carried out between November and December 2019. The experimental rats were randomly divided into three groups (control group and groups receiving 0.05 ml and 0.1 ml of BCG, respectively). To evaluate the effect of BCG on WBC differential count (total leucocytes and lymphocytes), RBC count (RBC count and PCV), and platelet count. **Results:** The results revealed a statistically significant variation among the groups, particularly in body weight and erythrocyte count. Rats administered 0.05 ml of BCG exhibited a significant increase in body weight (223.00 ± 9.5 g) compared to the control (195.50 ± 32.9 g) and the 0.1 ml BCG group (176.60 ± 11.6 g). Although total leukocyte counts did not differ significantly among groups, erythrocyte counts were significantly elevated in the 0.1 ml BCG group ($8.1800 ± 0.49 \times 10^6$/mm^3), indicating enhanced erythropoietic activity. Furthermore, lymphocyte percentages were highest in the 0.05 ml group (82.600 ± 4.0%), suggesting a dose-responsive immunostimulatory effect. However, a reduction in thrombocyte levels was noted with increasing BCG dosage, with the 0.1 ml group showing the lowest count (404.20 ± 121.3/mm^3). **Conclusion:** These findings suggest that a 0.05 mL dose of BCG may positively influence body weight gain and immune cell activity.

Keywords: BCG, Rats; Hematological parameters; Vaccine; Tuberculosis

1. Introduction:

Bacillus Calmette–Guérin (BCG) is one of the oldest vaccines still in widespread use and remains a cornerstone of tuberculosis (TB) prevention programs worldwide. Developed from an attenuated strain of *Mycobacterium bovis*, the BCG vaccine has been administered for nearly a century and is given to more than 160 million individuals annually, particularly in countries with a high burden of tuberculosis [1]. Although BCG provides inconsistent protection against adult pulmonary TB, it is highly effective in preventing severe and disseminated forms of the disease in infants and young children, including miliary TB and tuberculous meningitis [2].

The protective efficacy of BCG against pulmonary tuberculosis varies widely, ranging from no measurable protection to approximately 80%, depending on geographic region, population characteristics, and environmental factors [2]. Despite this variability, BCG vaccination has been shown to elicit strong cellular immune responses, particularly in neonates and young children. Several studies have demonstrated that BCG induces T-helper 1 (Th1) immune responses characterized by the production of interferon-gamma, which plays a critical role in host defense against mycobacterial infections [3].

Tuberculosis continues to pose a major global health challenge. Each year, *Mycobacterium tuberculosis* causes millions of new infections and remains one of the leading causes of death from infectious diseases worldwide [4]. The World Health Organization estimates that more than 10 million new TB cases occur annually, with approximately 1.5–1.8 million deaths reported each year [5, 6]. Despite advances in diagnosis and treatment, gaps in healthcare access, delayed case detection, and the emergence of drug-resistant strains have hindered global TB control efforts [7]. These challenges underscore the ongoing importance of vaccination strategies and the need to better understand the biological effects of existing vaccines, such as BCG.

Beyond its role in TB prevention, BCG vaccination has been associated with broader immunological effects, including modulation of both innate and adaptive immune responses. Hematological parameters, such as red and white blood cell counts and platelet levels, are closely linked to immune function and can provide valuable insight into systemic immune activation. In vertebrates, red blood cells have been shown to participate in immune responses to pathogens, while leukocytes play a central role in host defense against bacterial and viral infections. Platelets, traditionally associated with

hemostasis, are now recognized as active participants in innate immunity and inflammatory processes [8].

Changes in hematological parameters following vaccination may therefore reflect underlying immunostimulatory effects. Experimental animal models, including albino rats, provide a controlled setting for examining these changes and allow for detailed assessment of vaccine-related physiological responses. However, data on the hematological effects of BCG vaccination remain limited, particularly in experimental settings relevant to developing countries.

To date, no published studies from Yemen have examined the impact of BCG vaccination on hematological parameters in animal models. This represents an important gap in the regional scientific literature. Accordingly, the present study aimed to evaluate the effects of different doses of the BCG vaccine on selected hematological parameters in albino rats. Specifically, the study assessed changes in red blood cell count, packed cell volume, white blood cell count, lymphocyte percentage, platelet count, and body weight following vaccination.

In addition to contributing experimental data, this study seeks to address common misconceptions within the community, particularly concerns that vaccines may negatively affect growth, blood health, or immune function. By providing evidence from a controlled laboratory study, the findings may help strengthen public confidence in vaccination and support the continued use of BCG as a safe and effective immunization tool.

2. Materials and methods

2.1 Experimental Animals

The study was performed on eighteen male albino rats. The rats were 6 to 8 weeks old and obtained from the animal house of the Faculty of Applied Sciences at Sana'a University, Yemen. The rats were housed individually in stainless steel cages. The rats were kept in a temperature-controlled room ($21 \pm 2 °C$), and a 12-hour light/dark cycle was maintained [9]. Rats are fed a standard diet and water during the adaptation period and experimentation [10].

2.2 Animal Feeding

The diet formula consists of corn 30%, soybean meal 8%, wheat bran 7%, wheat 25%, dried fish (called Wazef locally) as a source of animal protein 10%, Sorghum Stover 20%, and 1 teaspoon (3.5 kg) of the above stock of vegetable oil. The ingredients were ground, mixed together, and supplemented with multivitamins and minerals (0.5 g per 5 L). The yielded paste was rolled into cylindrical pellets and dried. Each rat received approximately 100 g/day of dried pellets. Water was supplied [11, 12]. All rats were allowed to access water and diet freely [13].

2.3 BCG Vaccine

Freeze-dried (Danish 1331) was obtained from the branch of the Public Health and Population Office in Dhamar district, Dhamar governorate. BCG is a live, attenuated vaccine (Bacillus Calmette-Guérin strain) that is reconstituted with sodium chloride injection. **(Serum Institute of India Pvt. Ltd.).** The freeze-dried vaccine (each 0.1 ml contains between 2×10^5 and 8×10^5 C.F.U.) was reconstituted in diluents immediately before vaccination [14].

2.4 Methods

2.4.1 Experimental Design

The study was performed on 18 male albino rats, which were rested and allowed to adapt for 7–10 days before the experiment. The rats were divided into three groups, with equal random assignment to each group (6 rats per group). Body weights were recorded for all rats at the beginning and at the end of the experimental period to monitor any changes associated with the treatment. The experimental rats were divided randomly into three groups, and the hematological tests were as follows:

- **Control group:** 6 male albino rats were given equal volumes of normal saline.
- **Immunized group of 0.05 ml of BCG vaccine:** 6 male albino rats were submitted for hematological tests after receiving 0.05 ml of BCG vaccine. BCG was injected intradermally in the back with 50 μl/rat of BCG suspension containing 10^5 colony-forming units (CFU) according to a previously described procedure.
- **Immunized group of 0.1 ml of BCG vaccine:** 6 male albino rats were submitted for hematological tests after being immunized with 0.1 ml of BCG vaccine.

2.4.2 Specimens' Collection

Specimen collection during 2-3 weeks after vaccination. Blood specimens were collected using disposable syringes. The amount of blood collected with EDTA tubes, with gentle mixing for the hematological parameters assay, was analyzed within 2 hours of collection.

2.4.3 Assay

Hematological parameters

Total red and leucocyte counts were analyzed using an automated hematological analyzer (Nihon Kohden, Jaban). Parameters reported included erythrocytes, packed cell volume (PCV), leukocyte totals (TLC), lymphocytes, and platelets. The hematological parameters were analyzed at Dubai Specialized Labs, Dhamar City, Yemen.

2.4.4 Data Analysis

The data was analyzed by normal distribution using Kolmogorov-Smirnov. The results showed that the data were distributed normally, and then after that, the data were analyzed by one-way ANOVA. The results are expressed as means ± SD. The comparison between the control and the groups. The means were compared using Duncan's method in SAS 9.1.3. program.

3. Results

Tables 1 and 2 show the pre-experiment and post-treatment body weights (in grams) of rats in three groups: control, 0.05 ml BCG, and 0.1 ml BCG. In Table 1, there are no significant differences in pre-experiment weights, with the control group averaging Mean ± SD 129.50 ± 32.0 g, the 0.05 ml BCG group 128.00 ± 15.7 g, and the 0.1 ml BCG group 136.20 ± 17.4 g, as indicated by the same superscript (a). However, there is a significant difference in post-treatment, with the 0.05 ml BCG group showing the highest weight gain (Mean ± SD 223.00 ± 9.5 g), significantly higher than the 0.1 ml BCG group (Mean ± SD 176.60 ± 11.6 g) and the control group (Mean ± SD 195.50 ± 32.9 g), as indicated by different superscripts. These findings suggest that the 0.05 ml BCG dose leads to the most significant weight increase.

Table 1: Mean ± SD of Pre-Experiment and Post-Treatment Body Weights in Rats Following BCG Vaccine Administration.

Groups	N	Pre-experiment Mean ± SD (g)	Post-treatment Mean ± SD (g)
Control group	6	129.50 ± 32[a]	195.50 ± 32.9[ab]
0.05 ml of the BCG group	6	128.00 ± 15.7[a]	223.00 ± 9.5[a]
0.1 ml of the BCG group	6	136.20 ± 17.4[a]	176.60 ± 11.6[b]

(The values with different letters mean significant differences)

Table 2 summarizes the hematological parameters (%) in rats after BCG vaccination, with statistical significance indicated by the superscript letters. The 0.1 ml BCG group had the highest RBC count ($8.1800 \pm 0.49 \times 10^6$/mm³), followed by the control group ($7.6650 \pm 0.81 \times 10^6$/mm³). The 0.05 ml BCG group had a significantly lower RBC count ($7.2280 \pm 0.67 \times 10^6$/mm³), marked by superscript b. For packed cell volume (PCV), no significant differences were observed across the groups. Platelet counts were highest in the control group (Mean ±STD $520.83 \pm 53.7 \times 10^3$/mm³), and the 0.05 ml BCG group (Mean ±STD $461.60 \pm 57.3 \times 10^3$/mm³), but the 0.1 ml BCG group had a significantly lower platelet count (Mean ±STD $404.20 \pm 121.3 \times 10^3$/mm³), marked by superscript b. White blood cell (WBC) counts showed no significant differences between the groups. Finally, lymphocytes, the 0.05 ml BCG group (Mean ± STD 82.600 ± 4.0%) had a significantly higher percentage than the control group (Mean ± STD 73.833 ± 5.1%). In contrast, the 0.1 ml BCG group (Mean ±STD 74.800 ± 3.3%) showed no significant difference from the control group. The results of this study show that the BCG vaccine, at varying doses, leads to dose-dependent alterations in hematological parameters, with significant differences in RBC count, platelet count, and lymphocyte percentage.

Table 2: Mean hematological parameters (%) in rats following treatment with the BCG vaccine.

Groups	N	RBC (×10^6/mm^3) ± STD	PCV (%) ± STD	Platelets (×10^3/mm^3) ± STD	WBC (×10^9/L) ± STD	Lymphocytes (%) ± STD
Control group	6	7.6650 ± 0.81[ab]	49.850 ± 4.7[a]	520.83 ± 53.7[a]	8.73±3.0[a]	73.833 ± 5.1[b]
0.05 ml of the BCG group	6	7.2280 ± 0.67[b]	46.900 ± 4.3[a]	461.60 ± 57.3[ab]	9.180 ±3.2[a]	82.600 ± 4.0[a]
0.1 ml of the BCG group	6	8.1800 ± 0.49[a]	52.080 ± 3.7[a]	404.20 ±121.3[b]	10.0 ±2.1[a]	74.800± 3.3[b]

(The values with different letters mean significant differences)

4. Discussion

The highest weight gain was observed with the administration of 0.05 ml of BCG vaccine, indicating that this dose is the most effective for promoting weight increase. This study was in agreement with Fisker et al. (2011), whose results showed that administering the BCG vaccine at birth appears to be beneficial for boys' growth. Perhaps the BCG vaccine played a significant role in weight gain, as this study has demonstrated [15]. This may suggest that the 0.05 ml dose is optimal for stimulating the immune system and activating metabolic pathways that promote weight gain, while effectively balancing immune activation with more efficient nutrient utilization.

Among all groups, the 0.1 ml (100 µl) BCG group showed the highest mean red blood cell (RBC) count of 8.1800 ± 0.49, and this concentration was associated with a significantly greater increase in RBC count than the 50 µl BCG group. This effect may be due to the negative impact of the higher BCG vaccine dose, which could lead to loss of appetite and decreased fluid intake, resulting in dehydration. According to Pagana K and Pagana T (2014), dehydration can significantly increase RBC counts [16]. This study aligns with the findings of Silitonga M and Silitonga PM (2017), which indicate that RBC counts tend to increase in all AEP treatments but may also rise again when BCG is administered [8]. No statistically significant difference in mean packed cell volume was observed across groups, indicating that the treatment conditions did not have a measurable impact on this parameter. Similar to these findings by Silitonga M and Silitonga PM (2017), who found no significant difference in AEP and BCG in rats with control, all of them were in the normal range [8].

Platelet counts were highest in the control group, followed by the 0.05 ml BCG group, with the lowest counts observed in the 0.1 ml BCG group. Another study supports this finding, where the dose of BCG administered appears to correlate with the severity of thrombocytopenia (low platelet count). They found that a high dose of BCG (5 × 10^8 viable organisms) resulted in severe thrombocytopenia in two patients, with platelet counts falling below 2,000 and persisting for two weeks [17]. This study was in disagreement with the study by Silitonga M and Silitonga PM (2017), who indicated that the PLT increased significantly in all treatments compared to the controls [8].

The study revealed that the mean total white blood cell count was comparable across all groups. The reason may be that the difference was in the differential count and not in the TLC. This finding aligns with Jensen SK et al. (2020), who reported no significant differences in WBC counts between the BCG and control groups, suggesting that BCG does not affect WBC levels in healthy infants [18] . On the other hand, this study disagreed with the study by Silitonga M, Silitonga PM (2017), who found that white blood cells increased significantly in treatment AEP (31.5 g/kg body weight, 31.5 g AEP/kg body weight + BCG) [8].

The analysis identified significant variations in lymphocyte counts, with the 0.05 ml BCG group showing a marked increase compared to both the control and 0.1 ml BCG groups. The elevated lymphocyte levels may be attributed to a cellular immune response triggered by the BCG vaccine. These findings are consistent with previous research demonstrating that BCG vaccination of low-birth-weight neonates in West Africa increased in vitro cytokine responses to both specific and nonspecific stimuli. BCG vaccination enhanced Th1-polarizing cytokine responses (IFN-γ), shifted the TNF-α: IL-10 ratio toward more pro-inflammatory responses, and stimulated greater production of cytokines. Furthermore, BCG was found to be particularly effective in stimulating CD4+ T lymphocytes [19]. Our study supports a trial on neonatal BCG, which found limited effects on lymphocyte subsets, including an increase in effector memory cells [20]. However, disagree with the study by Jensen SK et al. (2020), who found no significant differences in WBC differential counts between the BCG and control groups [18].

5. Conclusions and Recommendations

In conclusion, the administration of BCG at varying doses elicited notable hematological responses in rats. The 0.05 ml dose was associated with favorable physiological and immunological outcomes, including increased body weight and a significant rise in lymphocyte percentages, suggesting enhanced immune activation without adverse hematological effects. Conversely, the 0.1 ml dose, although effective in elevating erythrocyte count, resulted in decreased thrombocyte levels and body weight, which may indicate dose-dependent hematotoxicity or systemic stress. These findings underscore the critical importance of dose optimization to prevent adverse systemic effects.

Based on the study's findings, we recommend the following: prioritizing the BCG vaccine for its proven immune response; conducting further research on its components, adjuvants, and varying doses' effects on platelet indices; ensuring the provision of necessary resources for studies on cell-mediated and humoral immunity, as well as cytokine changes; investigating other vaccines entering the country; and conducting comparative studies on BCG vaccines from different manufacturers to assess efficacy variations.

Ethical Approval

The animal protocol in this study complied with the Guide for the Care and Use of Laboratory Animals. The BCG vaccine was obtained from the Public Health and Population Office branch in Dhamar, Dhamar Governorate. The BCG vaccine is stored at the proper temperature. The study was conducted after obtaining approval from the Biology/Microbiology Department of the Faculty of Applied Science at Thamar University.

Data Availability

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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