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RESEARCH PAPER

CD4 AND CD8 COUNTS OF BACILLUS CALMETTE GUERIN (BCG) VACCINATED NEONATES IN PARTS OF EDO AND DELTA STATES, NIGERIA

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ABSTRACT

This study examines the cellular immune factors responsible for combating infections by assessing CD4 and CD8 counts of neonates (pre and post BCG vaccination). A total of 373 blood samples were collected from neonates that visited the immunization clinics at Irrua Specialist Teaching Hospital (ISTH), Irrua and Federal Medical Centre (FMC), Asaba, Nigeria. CD4 and CD8 easy count kit (Partec, Germany) was used for the determination of CD4 and CD8 count respectively, while the samples were analysed using SL-blue Cyflow. At ISTH Irrua, 191 samples were analyzed (130:60; pre and post vaccination), while at Asaba, 182 samples were analysed (120:62; pre and post vaccination). The results showed that CD4 count was significantly higher for Pre vaccination than Post vaccination at both locations. At FMC Asaba, the CD4 count for females was significantly higher than in males (pre-BCG vaccination), while CD4 count was not significantly affected by gender at Irrua, ISTH. CD8 increases in both locations but was not significantly affected by gender. The findings of this study therefore suggests that there is a cell mediated immune response to BCG vaccine by both the male and female neonates and this is associated with a decrease in CD4 count (post vaccination).

Keywords: Edo, Delta, Male, Female, Neonates, BCG, CD4, CD8

INTRODUCTION

Tuberculosis is a major public health disease and has been described as humanity's greatest killer (WHO, 2002; Cadmus *et al*., 2011). The disease is increasing at an alarming rate primarily due to the absence of an effective vaccine, the emergence of multi-drug resistant strains, as well as co-infection with HIV and low diagnostic and therapeutic coverage (Acosta *et al*., 2010).

Bacillus Calmette Guerin (BCG) is the only available vaccine against tuberculosis and has been in use for over seventy years (Pinto *et al*, 2004; Kassyam *et al*, 2010). BCG consist of an attenuated strain of *Mycobacterium bovis*, which has been reported with varied efficacy (0–80%) depending on the studies and geographical locations (Glassroth, 1997, Brodin *et al*, 2004; Pinto *et al*, 2004). In spite of this, more people have been vaccinated with BCG than any other vaccine (Ota *et al*, 2002). Although the efficacy of BCG vaccine against the disease in adult is variable, it protects against childhood disease (Ota *et al*, 2002). Presently in Nigeria, BCG is administered to children at birth (one dose of 0.05ml) and it is usually not administered to children who have clinical HIV/AIDS (NPI, 2004).

Mycobacterial infections induce humoral and cell mediated immune responses, in which the contribution of macrophages, CD4 and CD8 T-lymphocytes is essential (Levison and Jawetz, 1998; Gil *et al*, 2004). Both animal and human data support the key role of CD4 T-cells in the control of *Mycobacterium tuberculosis* (Chackerian *et* al., 2001). Greater morbidity and mortality in CD4 depleted mice confirm the critical role of this T-cell subset (Chackerian *et al*, 2001). The resurgence of tuberculosis associated with HIV epidemic also demonstrates that loss of CD4 T-cells increases susceptibility to tuberculosis (Sonneberg et al, 2001).

The aim of this study therefore, is to determine the CD4 and CD8 counts of neonate's (pre and post vaccination-six weeks after BCG was administered) with the objective of determining the gender differences.

MATERIALS AND METHODS

Study Area: The study was conducted at the Irrua Specialist Teaching Hospital (ISTH) Irrua and Federal Medical Centre (FMC) Asaba, both in Nigeria. Irrua is the administrative headquarters of Esan Central Local Government Area, Edo State and ISTH is a designated centre for the National Programme for Immunization (NPI, 2004), where routine immunization is rendered to children 0 to 59 months.

Asaba on the other hand, is the state Capital of Delta State and is located on the right bank of the river Niger. The Federal Medical Centre in Asaba renders routine immunization to children 0 – 59 months within the city and other neighbouring towns.

Inclusion criteria: The study targets children 0 to 28 days old (neonates) who were born in these hospitals and registered in the immunization clinic at the time of this study.

Exclusion criteria: Children that were 30 days old and above, pre-termed babies and those born with aliments were excluded in this study.

Ethical consideration: Ethical approval was sought and granted by the research and ethic committee of ISTH, Irrua and FMC Asaba, while informed consent was obtained from the parents of the neonates.

Duration of Study: The study was conducted within a six months period (June to December, 2011).

Study Design: The study adopted the cohort study design.

Method of sample collection: A total of three hundred and seventy (373) blood samples were collected from neonates attending immunization clinics at Irrua Specialist Teaching Hospital, Irrua and Federal Medical Centre, Asaba. The samples were collected by pediatricians using 21G and 23G needles into EDTA vacutainers. 1ml of venous blood was collected from each neonate before BCG vaccine was administered (Pre-BCG vaccination) and six (6) weeks after BCG was administered (Post-BCG vaccination). The samples were transported to the laboratory in geostyles (vaccine carriers) containing frozen ice – packs.

Method of Sample analysis: The CD4 % easy count partec kit (05 – 8405) and CD8 easy count partec kit (05 – 8801) were used for the determination of CD4 and CD8 counts respectively.

In analyzing for CD4, 20µl of whole blood was dispensed into a corresponding partec test tube and 10µl of CD4 monoclonal ABPE and 10µl of CD45 monoclonal ABPE – DY647 was added. The contents were properly mixed and incubated at room temperature in the dark for 15 minutes. After which, 400µl of buffer 1 was added and mixed. 400µl of buffer 2 was added mixed and each sample was analyzed immediately using SL – blue Partec cyflow (Balakrishnan *et al.,* 2005).

CD8 count was determined by dispensing 20µl of whole blood into Partec tube and adding 20µl of CD8 m ABPE. The contents was gently mixed and incubated at room temperature in the dark for 15 minutes, 800µl of buffer was added, properly mixed and each sample was analyzed using SL – blue Partec cyflow (Ray *et al.,* 2006).

Data analysis: Statistical analysis was done using the student "t" test to determine level of significance. A p- value of less than or equal to 0.05 ($P \leq 0.05$) was considered to be statistically significant.

RESULTS

The effect of BCG vaccination on CD4 and CD8 count in Irrua and Asaba is shown in Figures 1 and 2 respectively. CD4 count was significantly higher pre BCG vaccination than CD4 count post vaccination in both locations. CD8 count increased at both locations post BCG vaccination. Figure 3 shows the effect of BCG vaccination on CD4 count of male and female neonates at Irrua and Asaba, while Fig 4 shows the effect of BCG vaccination on CD8 count of male and female neonates in Asaba and Irrua. In Asaba, CD4 count for females was significantly higher than in males pre BCG vaccination ($P < 0.05$). On the other hand, CD4 count was not significantly affected by gender at Irrua. CD8 count was not significantly affected by gender at both locations.

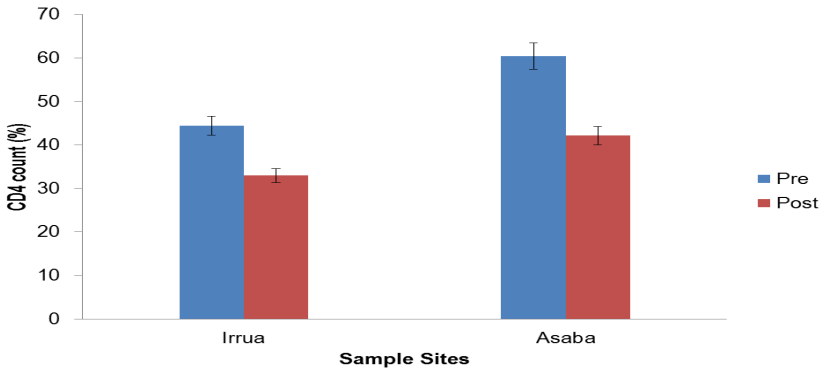

Figure 1: Effect of BCG vaccination on CD4 count in Irrua and Asaba

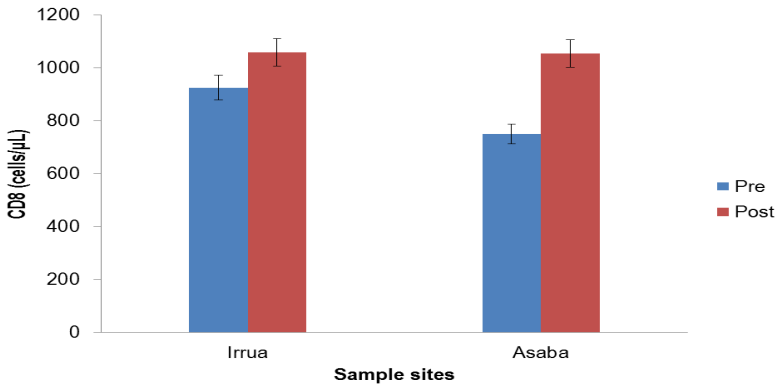

Figure 2: Effect of BCG vaccination on CD8 count

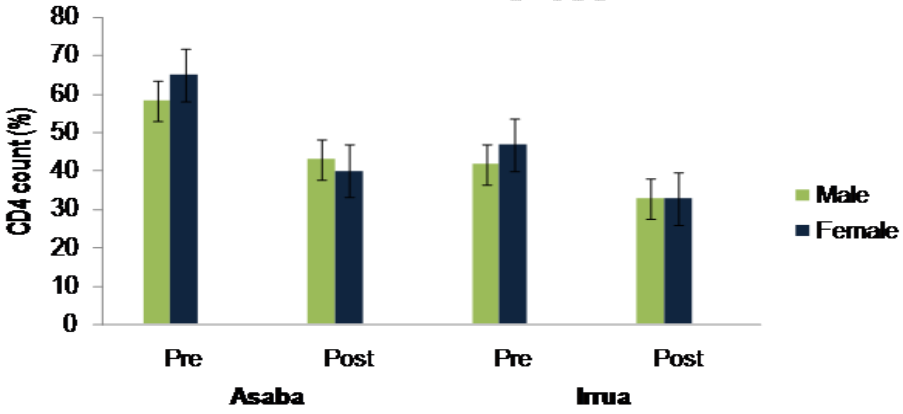

Figure 3: CD4 count of male and female infants in Asaba and Irrua

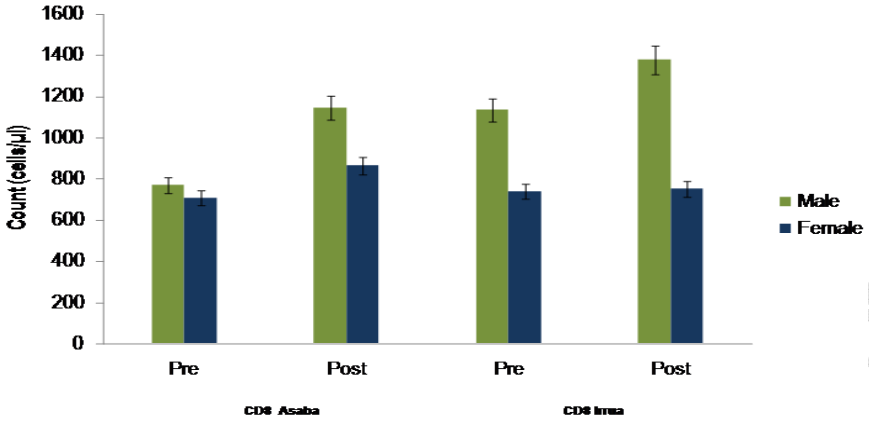

Figure 4: CD8 count of male and female infants in Asaba and Irrua

DISCUSSION

CD4 count (pre BCG-vaccination) was significantly higher than CD4 count (post vaccination) at both locations ($p<0.05$). This decrease is at variance with studies done by Hanekom (2005), where there was quantitative increase in the proliferation of CD4 cells. However Kagina *et al*. (2009) reports that CD4 T cell response is heightened when BCG is given at 10 weeks instead of at birth. In this study, BCG was administered at birth.

Also, the observed increase in CD8 count at both locations agrees with the studies by Hanekom (2005), where CD8 T cells with cytotoxic potential were produced after BCG vaccination, though CD8 T cell proliferation was quantitatively lower than that of CD4 Tcells.

Available literature show that *mycobacterium* induces CD8 T cell responses in humans (Flynn et al., 2004) and large body of evidence supports a role for CD8 T cells in protection against tuberculosis (Grotzke and Lewinsohn, 2005). However, CD4 T cells are more important in acute infection whereas CD8 T cells are responsible for the containment of latent infection (Van Pinxteren *et* al., 2000).

In Asaba, CD4 count of females was significantly higher than in males (pre-BCG vaccination), but was not significantly affected by gender at Irrua. Males had significantly higher levels of CD8 counts than females (pre and post BCG vaccination) at Irrua, while at Asaba, CD8 count was not significantly affected by gender. Although Benn and Aaby (2007) asserts that gender can affect immune response to BCG vaccine, Wilson *et al* (1995) reported however, that many factors may influence host response to BCG vaccine. These factors include socio-economic conditions, genetic composition of the population, climate, exposure to sunlight, diet and nutrition.

The findings of this study therefore, have shown that gender is just one of the many factors that can affect response to BCG vaccine by neonates. Furthermore, it has also revealed that there is cell mediated immune response to the BCG vaccines administered to Nigerian children at birth in parts of Edo and Delta States.

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AUTHOR(S) CONTRIBUTION

Eyaufe A.A, is the principal investigator while Esumeh F.E. was involved in the design of the study. Alika S. and Adeniran K. were involved in selection of subjects and sample collection. Festus O.O and Osagie R.N. were involved in sample storage and statistical analysis.