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TNF ALPHA LEVELS OF BCG VACCINATED NEONATES IN PARTS OF EDO AND DELTA STATE, NIGERIA

Eyaufe A. A. O.,*[1] Esumeh F. I.,[2] Osagie R. N.,[1] Adeniran K.,[3] Alikah S.,[4] Festus O. O.[5], Iserhienihien O.[6], Unuabonah F. H.[5] and Okodua M. A.[5]

[1]Department of Medical Microbiology, Ambrose Alli University, Ekpoma, Edo State, Nigeria.
[2]Department of Microbiology, Ambrose Alli University, Ekpoma, Edo State, Nigeria.
[3]Department of Pediatrics, Federal Medical Centre, Asaba, Delta State, Nigeria.
[4]Department of Pediatrics, Irrua Specialist Teaching Hospital, Irrua, Edo State, Nigeria.
[5]Department of Medical Laboratory Science, Ambrose Alli University, Ekpoma, Edo State, Nigeria.
[6]Department of Community Medicine, Ambrose Alli University, Ekpoma, Edo State, Nigeria.

***Corresponding Author: Dr. Eyaufe A. A. O.**

Department of Medical Microbiology, Ambrose Alli University, Ekpoma, Edo State, Nigeria.

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ABSTRACT

This study assesses BCG uptake by determining quantitatively TNF alpha levels pre(before BCG was administered) and post BCG vaccination(six weeks after BCG was administered). The cytokine, tumor necrosis factor alpha (TNFα) plays a paramount role in curtailing infection by *Mycobacterium tuberculosis*, the causative agent of tuberculosis in humans. M. tuberculosis can counter this TNFα based defense by decreasing host cell TNF production. Three hundred and seventy three serum samples were analyzed for TNF α using the human TNF ELISA kit (Boster Biological Technology Co. Ltd, USA). In Irrua, 191 samples (130 pre- BCG vaccination and 61 post-vaccination) were analyzed, while in Asaba, 182 samples (120 pre-BCG vaccination and 62 post BCG vaccination) were analyzed. In Asaba, TNFα levels was the same pre-BCG vaccination in both centres. Post vaccination, TNFα levels in Asaba was significantly higher ($P < 0.05$). There was no significant difference in TNFα levels of male and female infants pre and post BCG vaccinations. There was weak correlation between age and TNF alpha levels. The decrease in TNF levels post BCG vaccination reveals that, the vaccine did not enhance TNFα levels, which is crucial in Mycobacterium tuberculosis infection. Thus it is necessary to develop tuberculosis vaccines that can enhance TNFα production. This study also shows that in neonates, age and gender did not affect TNF alpha response.

KEYWORDS: TNFα, BCG, Neonates.

INTRODUCTION

Tuberculosis remains a major global public health burden and the disease is still on the increase due to the absence of an effective vaccine, the emergence of multi – drug resistant strains as well as co – infection with HIV coupled with low diagnostic and therapeutic coverage (WHO, 2002; Acosta *et al*., 2010, Cadmus *et al.*, 2011). The bacilli of Calmelite and Guerin (BCG), which was introduced to developing countries in 1950's is the only available vaccine against tuberculosis given to children in Nigeria as a single dose at birth. (NPI., 1999; Fine *et al*., 2004). Although the efficacy of BCG vaccine against the disease in adult is variable, it protects against the childhood disease (Ota *et al.*, 2002).

UNICEF is the largest supplier of BCG vaccines distributing more than 120 million doses each year to more than 100 countries (Ritz *et al*., 2008). Worldwide over 90% of children are immunized with BCG making it the most commonly administered vaccine, with more than 120 million doses used each year. Tuberculosis is a well – recognized problem in homeless people whose number has increased particularly in Africa (Ankrah, 1997). According to the Nigerian tuberculosis fact sheet by the United States embassy in Nigeria, Nigeria ranks 10th among the 22 high – burden 713 countries in the world. WHO estimates that 210,000 new cases of all forms of TB occurred in the country in 2010 equivalent to 133/100,000 cases.

Tumor necrosis factor (TNFα) is an inflammatory mediator released primarily by macrophages. It has many important effects that differ depending on the concentration. At low concentration, it increases the synthesis of adhension molecules by endothelial cells, which allows neutrophils to adhere to blood vessel walls at the site of infection. It also activates the respiratory burst within neutrophils, thereby enhancing the killing power of these phagocytes. It increases lymphokine synthesis by helper T-cells and stimulates the growth of

B-cells. Kaneko *et al*., (1999) and Saunders *et al.*, (2005) have reported that TNFα is present at sites of active *Mycobacterium tuberculosis* infection in humans, regardless of the stage of mycobacterial infection. Studies by Ehlers (2003) in mouse infection models demonstrates that TNF alpha is a critical component of both the antibacterial protective and the inflammatory immune response to *Mycobacterium tuberculosis.* TNFα play an essential role in preventing reactivation of persistent tuberculosis, modulates the pulmonic expression of specific immunologic factors and limits the pathological response of the host (Mohan *et al*., 2011; Basingnaa *et al*., 2019).

MATERIALS AND METHODS

Area of study

Immunization clinics of Irrua Specialist Teaching Hospital Irrua and Federal Medical Centre, Asaba in Edo and Delta States respectively of Nigeria, which are designated centres for routine immunization. Irrua is the administrative headquarters of Esan Central Local Government Area and has a total population of 189, 316 (NPC, 2006). Irrua has a Federal Teaching and specialist Hospital, which is a designated centre for the National programme for Immunization (NPI) where routine immunization is administered to children 0 – 59 months. Asaba is located on the right bank of the River Niger and has a Federal Medical Centre, which renders routine immunization to children 0 – 59 months within the city and other neighboring towns and cities.

Sample size

The study comprised of 250 subjects registered in the immunization clinics of Irrua Specialist Teaching Hospital, Irrua and Federal Medical Centre, Asaba.

Statistical analysis

This was done using the student "t" test to determine the level of significance and correlation analysis. A P-value of less than or equal to 0.05 ($P < 0.05$) was considered to be statistically significant.

Exclusion and Inclusion Criteria

Samples were collected only from healthy neonates who registered in the immunization clinics of these health institutions. The choice of babies for this study was based on the fact that BCG is given at birth.

Ethical Consideration

Informed consent was obtained from mother while ethical approval was sought from the Research and Ethics committee of the health institutions.

Sample collection/ analysis

Sample were collected twice, before BCG was administered and six weeks after BCG was administered. Two (2) mls of venous blood was collected from selected subjects into plain vacutainers using 21 G and 23 G sterile needles. 0.05ml of BCG vaccines was injected intradermally into the upper left arm of the neonates after cleaning with sterile wet cotton wool swabs. Two (2) mls of blood in plain vacutainers were centrifuged at 3,000 rpm for 5 minutes and supernatant (serum) removed using eppendorf automatic pipettes into another set of plain properly labeled vacutainers. The serum samples were assayed for TNF alpha using the bosters human TNF ELISA kit (Eko525, Boster Biological Technology Co. Ltd USA). The sample were analyzed according to manufacturers instruction.

RESULTS

Figure 1 shows TNFα levels in Pre and post BCG vaccinated Children in Irrua and Asaba. TNFα levels was the same pre-BCG vaccination in both centres. Post vaccination TNFα levels in FMC Asaba was significantly higher. Figure 2 represents TNFα levels of male and female children pre and post BCG vaccination. There was no significant difference in TNFα levels of male and female infants pre and post BCG vaccination. The correlation of age and TNFα levels pre and post BCG vaccination are shown in figures 3 and 4.

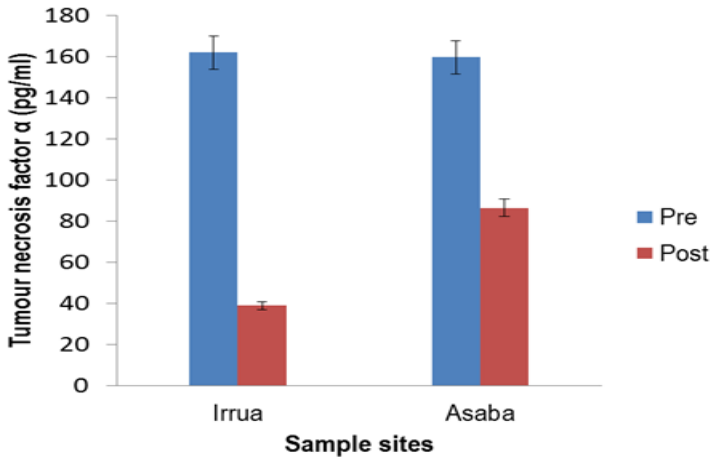

Figure 1: Effect of BCG vaccination on TNF α levels.

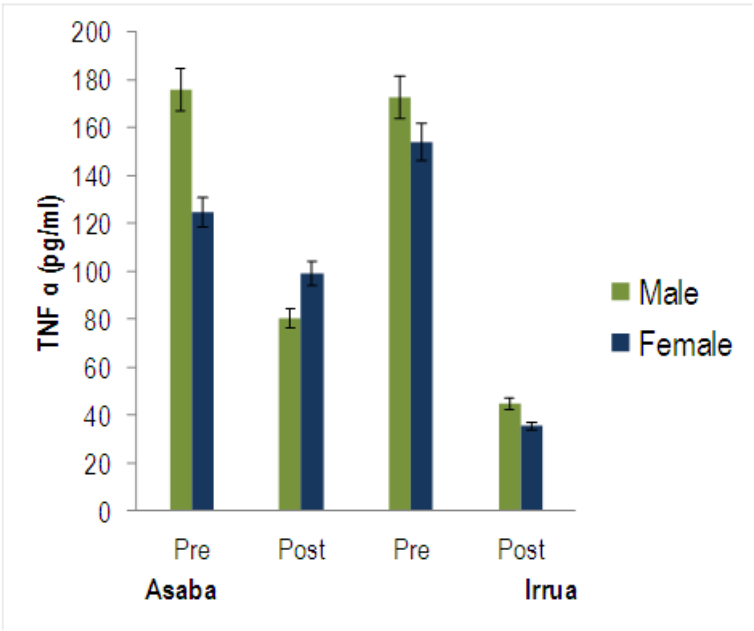

Figure 2: Tumour necrosis factor α levels of male and female infants in Asaba and Irrua.

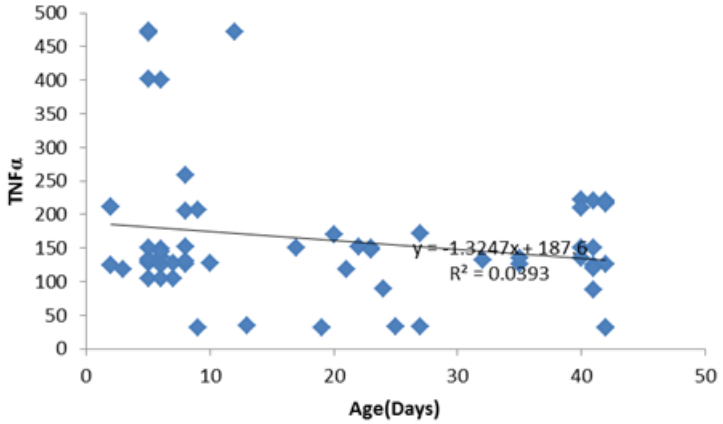

Figure 3: Correlation of age and pre TNFα in Irrua.

DISCUSSION

TNF alpha levels of 250 neonates were assessed before BCG was administered, of which 123 of them reported six weeks later to be assessed post-BCG vaccination. TNF plays a major role in the initial and long term control of tuberculosis. TNF is important in macrophage activation as well as recruitment to the site of infection. In humans treated with TNF – neutralizing drugs, an increased susceptibility to tuberculosis as well as other infectious diseases were observed. TNFα levels was the same pre BCG vaccination in both centres. TNF alpha levels decreased in both centres, although post vaccination TNFα levels in Federal Medical Centre Asaba was significantly higher.

Mohan et al., (2001), Kaneko et al (1999) and Saunders et al., 2005 has reported that TNFα plays an important role in protective immunity against virulent mycobacteria. Islam et al (2004) has also reported that TNFα is present at sites of active *Mycobacterium tuberculosis* infection in humans, regardless of the stage of mycobacterial infection. Studies by Lasco *et al*., (2003) using guinea pig leukocyte population indicate that BCG vaccination enhances both proliferative and TNFα responses in guinea pig leukocyte populations. Mice are the most frequently used animal in studies of protective immunity induced by BCG immunization (Martin, 2006) although guinea pigs have also been commonly used to investigate the biological activity of BCG vaccines. However there are limitations when extrapolating findings from studies of the immune response in animal models to humans e.g cytokines such as TNFα can have different effects on mononuclear cells in different species (O'Garra and Britton, 2008).

Some studies have shown that attenuated strains of mycobacteria induce higher TNFα production in human macrophages (Keane et al., 1997 & Belton et al., 2000)

while other studies have also demonstrated the reverse to be possible (Tracy & Cerami 1992; Silver & Ellner, 1998). Olsen et al (2016) in their study identified *Mycobacterium tuberculosis* genes that can mediate inhibition of TNF production by macrophage. They knocked out some of these genes to produce *mycobacterium tuberculosis* mutants that can enhance macrophage TNF production and subsequent immunization of these mutants in mice triggered a -T-cell response stronger than elicited by the parental bacillus. Fatima *et al* (2016) in their study demonstrated significant serum levels of TNF alpha in patients with tuberculosis. This indicates, from our study that BCG cannot induce an effective TNF alpha response as *Mycobacterium tuberculosis*.

Tuberculosis still remains a global public health burden and immunization with an effective vaccine is one way to control tuberculosis. Tumor necrosis factor alpha plays an important role in controlling tuberculosis. The decrease in TNFα levels post BCG vaccination shows that the vaccine did not enhance TNFα levels which is crucial in *mycobacterium tuberculosis* infection. Therefore there is need to develop tuberculosis vaccines that can enhance macrophage TNF production.

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