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Original Article

TETANUS TOXOID ANTIBODY LEVEL IN ASYMPTOMATIC *PLASMODIUM FALCIPARUM* MALARIA PARASITEAMIC PREGNANT WOMEN.

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The present study was designed to investigate if the presence of asymptomatic malaria parasiteamia in pregnant women will compromise their ability to respond to full dose of tetanus toxoid immunization during their antenatal clinic visits. Hence, 90 apparently healthy pregnant women who had completed the tetanus toxoid immunization during the current pregnancy were recruited at the antenatal clinic and were divided into two groups based on the antenatal record of malaria paras during the immunization period. Sixty (66.7%) of the pregnant women were seroreactive for Plasmodium falciparum histidine rich protein- (HRP)-2 while 30 (33.3%) were seronegative for Plasmodium falciparum HRP-2. The malaria parasite density range for the seroreactive group was between 322 and 1045 parasites per ml of blood. The blood concentration of Tetanus toxoid antibody response in both groups of seroreactive and seronegative HRP-2 pregnant women did not show any significant difference in tetanus toxoid antibody response (p>O.2). This result showed that the presence of asymptomatic IPlasmodium falciparum malaria parasiteamia in the pregnant women during the immunization schedule did not compromise the ability to respond to tetanus toxoid immunization. Hence asymptomatic malaria may not contribute to the prevalence of neonatal tetanus in Nigeria, however, there is need to treat these pregnant women for asymptomatic malaria when detected in order to reduce the burden of malaria on them.

Key words: Tetanus toxoid, asymptomatic malaria, specific antibody, pregnant women.

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INTRODUCTION

Tetanus is considered a major health problem in the developing countries (Campbell, 2000). However, the numerous financial and human resources that have been invested into the eradication of neonatal tetanus have not yielded the desired goal in the developing countries. Since the effectiveness of tetanus toxoid immunization has been convincingly demonstrated in many field and hospital based studies (Newell et al 1971, Black et a! 1980, Cliff et al 1985, EPI 1988). It is necessary for extensive research to be conducted in developing countries experiencing high neonatal tetanus rates in order to discover the actual reason behind the observed results. Immunity against tetanus toxoid is antibody mediated and belongs to the IgG class of immunoglobulin (WHO/EPI). This could suggest that adequate immunization of the pregnant women may lead to effective protection of their neonates (WHO/EPI), through placental transfer of the antibody to the neonates. However, in Nigeria, pregnant women are not routinely screened for the presence of asymptomatic malaria parasiteamia and hence such pregnant women are not treated for malaria during pregnancy. However the recent report of high

incidence rate of asymptomatic malaria parasireamia amongst pregnant women in Nigeria (Onyenekwe, 2002), led to the design of this study in order to know the possible impact of this disease on one of the most prevalent immunization programmes in Nigeria. Therefore, the present study was designed to check the tetanus specific antibody levels in immunized pregnant women with antenatal record of asymptomatic malaria parasiteamia during the tetanus toxoid inununization schedules.

MATERIALS AND METHODS

Subjects:

90 apparently healthy pregnant women aged 19-4Oyears attending the antenatal clinic of Obstetric and Gynaecology Department were studied, These pregnant women had received fill dose of tetanus toxoid immunization in the course of their ahtenatal visits. After the immunization of the pregnant women was completed, they were divided into 2 groups based on the antenatal record of asymptomatic malaria parasiteamia during the immunization schedules. Sixty of the pregnant women had asymptomatic malaria parasiteamia and were reactive to *Plasmodium falciparum* }IRP-2 during the period of immunization. These were called positive subjects. The remaining 30 pregnant women had no asymptomatic malaria parasiteamia and were not reactive to *Plasmodium falciparum* HRP-2 during the period of immunization. These were called negative subjects. Malaria parasiteamia was used as the bases of dividing the pregnant women into groups because of the high prevalence of asymptomatic malaria parasiteaniia amongst pregnant women in Nigeria. Informed consent was obtained from the pregnant women and the University of Ibadan/University College Hospital, Board of Ethical Committee, Post-Graduate Institute for Medical Research and Training; Ibadan approved the design of this study.

Blood sample collection:

During the antenatal clinic visits by the pregnant women, asymptomatic *Plasmodium falciparum* malaria parasite screening was performed using *Plasmodium falciparum* HRP-2 seroreactivity and blood parasites detection and results of the investigation were recorded during the tetanus toxoid immunization. Two weeks after the full dose of tetanus toxoid immunization of the pregnant women, blood samples were collected by vene-puncture and dispensed into sterile containers. The separated sera were used for the determination of tetanus toxoid antibody level in the pregnant women.

This was done by *Plasmodium falciparum* histidine rich protein 2 seroreactivity based on indirect enzyme linked immunosorbent assay (Cellabs PTY Ltd Australia). The procedure as described by the manufacturers is briefly reported. Microwells were coated with monoclonal anti-*Plasmodium falciparum* histidine rich protein (HRP)-2. 100μl of test sample, reference positive control or reference negative control was added respectively to the coated wells. The wells were incubated at room temperature for 60 minutes to allow the fixing of the HIR antigen to the specific antibody coated to the wells. Other blood components were removed by washing each well with 0.01 M phosphate buffered saline (PBS)-Tween solution. The conjugate of enzyme labelled anti-human globulin was added to all the wells. This was allowed for incubation at room temperature for 60 minutes. The conjugate antibody will bind any antigen fixed to the well. The wells were washed as described above. The enzyme substrate solution was added and incubated in the dark for 15 minutes. This allows for colour development that indicates the presence of malaria antigen in the blood under test.

Interpretation of Result of histidine rich protein-2 showed that sixty pregnant women were sero-reactive to *Plasmodium falciparum* HRP-2 while the remaining 30 were sero negative to *Plasmodium falciparum* HRP-2.

Detection of Plasmodium falciparum parasites:

Plasmodium falciparum Malaria parasites were detected by microscopic examination of Giemsa stained thin and thick blood films and parasitaemia was expressed as malaria parasites density per ml of blood as described by Rooth et. Al (1991). The asymptomatic malaria parasites range detected in the *Plasmodium falciparum* HRP-2 sero-reactive pregnant women was between 322 and 1045 malaria parasites per microlitre of blood.

Determination of tetanus toxoid antibody:

The serum tetanus toxoid antibody concentration was determined by indirect enzyme linked immunosorbent assay. The procedure is briefly described. Microwells were coated with tetavax adsorbed tetanus vaccine (Pasteur Meriux, France). The serum samples for the determination of tetanus toxoid antibody were added in 100j volumes to the respective coated wells. The wells were incubated for 60 minutes at 37°c to allow the specific antibody to fix to the antigen. Other components not bound were washed off the wells with 0.OIM phosphate buffered saline (PBS)-Tween solution. The conjugate enzyme labelled anti-human globulin binds the tetanus antibody fixed to the well. Other conjugate antibody not bound was washed-off. 100μl of the enzyme substrate chromogen TMB (3,3,5,5-tetramethylbenzidine) was added to each well and incubated in the dark at room temperature for 15 minutes. The reaction was stopped with 2.5m1 HC1 and the absorbance of the colour developed was read on a dynatech MR 250 micro-plate reader (Guernsey Channel, Islands). Un-reacted well containing only the substrate solution and stopping reagent was used to blank the micro-plate reader. The absorbance was proportional to the concentration of the tetanus toxoid antibody in the serum.

Statistical analysis: This was performed using stac-pac Gold package. The mean (+lsd) was determined for the variables in each group while the analysis of variance was used to determine the level of significance between the variables. Level of significance was considered at p-value <0.05.

RESULTS

Table 1 shows the HRP-2 Seroreactivity rates and malaria parasites range (per ml of blood) among the tetanus toxoid immunized pregnant women. Sixty (66.7%) of the pregnant women were HRP-2 seroreactive while 30(33.3%) of the pregnant women were FIRP-2 seronegative. Similarly, the malaria parasites density range as observed in the HRP-2 seroreactive pregnant women was between 322 to 1045 parasites per ml of blood.

Table 1
Shows the HRP-2 Seroreactivity rates and malaria parasites density range (per ml of blood) among the tetanus toxoid immunized pregnant women

Malaria parasite status	Asymptomatic (positive) Pregnant women	Aparasiteamic (negative) Pregnant women
HRP-2 Seroreactivity	60 (66.7%) positive	30 (33.3%) negative
Malaria parasites density	322 <1045/mi of blood	Nil

HRP-2= Plasmodium falciparum histidine rich protein-2

Table 2 shows the mean (± 1 Sd) serum tetanus toxoid antibody concentration expressed as absorbance in the asymptomatic malaria parasiteamia positive and aparasiteamic negative groups of pregnant women immunized with tetanus toxoid.

The mean serum tetanus toxoid antibody absorbance was 0.710 ± 0,230 and 0.790 ± 0.220 in asymptomatic malaria parasiteamia positive and aparasiteamic negative groups respectively. There was no significant difference in mean tetanus toxoid antibody absorbance between the two groups (p>0.2).

Table 2:
Mean (±1sd) serum concentrations of tetanus toxoid specific antibody expressed in absorbance in asymptomatic malaria parasiteamia positive and negative pregnant women after full tetanus toxoid immunization.

Parameter	asymptomatic (Positive) pregnant women	aparasiteamic (Negative) pregnant women.	p-value
Tetanus toxoid antibody levels	0.710 ± 0.230 (n=60)	0.790 ± 0.220 (n=30)	p>0.2

sd = standard deviation

DISCUSSION

The present study observed the presence of asymptomatic *Plasmodium falciparum* positive pregnant women, thus confirming that pregnant women may also present with asymptomatic malaria. One of the possible reason for this phenomenon may be that the immune systems of the pregnant women was quite able to contain the attained blood malaria parasites density. Hence this study calls on concerned authority to include asymptomatic malaria parasites screening as part of normal antenatal routine checks in Nigeria and possible in other malaria endemic regions of the world.

An epidemiological implication of not treating asymptomatic malaria parasiteamia is that the infected pregnant women may serve as reservoirs for the spread of malaria to the community and probable to their feotus in utero. In addition, in the cases of stress or any condition that may suppress their inunune system, clinical presentation of malaria may occur easily due to the presence of several predisposing factors. Therefore the authors of the present study are of the opinion that asymptomatic malaria parasiteamia be treated during antenatal clinic visits as this will help to reduce the burden of malaria and possible chances of precipitation of clinical malaria during pregnancy.

Asymptomatic malaria parasiteamia is a type of malaria parasiteanua that is different from clinical malaria in presentation but in the presence of predisposing factors may precipitate clinical malaria in the individual. The individuals are known to present apparently healthy without any sign and symptoms of malaria. Characteristic of this pattern of parasiteamia is the maintenance of parasites threshold usually different from that observed in cases of acute or clinical malaria infections. It has been shown that single infection can persist for as long as 18 months in the absence of re-infection (Frank et al 2001, Krajden et al 1991). This could suggest the impact of such asymptomatic existence of infections on human and resources, The present study also observed that the asymptomatic *Plasmodium falciparum* malaria parasiteamia positive pregnant women on full tetanus toxoid immunization had similar tetanus toxoid specific antibody response as observed in the aparasitèamic negative pregnant women.

This result indicates that asymptomatic *Plasmodium falciparum* parasiteamia did not pose any immediate threat to achieving the desired target for tetanus toxoid immunization. Thus, there seem not to be any immunosuppressive effect of the presence of asymptomatic malaria parasiteamia on the specific response of infected pregnant women to tetanus toxoid immunization. In a study elsewhere, the antibody responses of malaria infected pregnant women to tetanus toxoid immunization was reported to be similar to that of non-pregnant healthy adults (Brabin et al 1984).

Increase in the prevalence of neonatal tetanus has been reported in Nigeria (Anti-Obong et al 1993, Owa and Makinde 1992). However, healthy Nigerian women and pregnant women on malaria chemoprophylaxis have been shown to respond adequately to tetanus toxoid immunization (Gilles 1983, Gini and Okafor 1992). This shows that generation of tetanus specific antibody is

not impaired in Nigeria women and could be assumed that their neonates would be protected. However, certain disease conditions, if present in a host, has been shown to induce immunosuppressive effect on the ability of the individual to respond to other infections. Possibly the incidence of any such disease may disrupt the ability of these pregnant women to generate adequate tetanus specific antibody and transfer of such antibody during pregnancy to their neonates (Gilles 1983, Gini and Okafor 1992).

Therefore the increase in prevalence of asymptomatic malaria in Nigerian pregnant women may have nothing to do with the increase cases of neonatal tetanus in Nigeria. The present study therefore suggests that the presence of asymptomatc *Plasmodium falciparum* malaria parasiteamia during tetanus toxoid immunization may not compromise the ability of the pregnant women to respond to tetanus toxoid immunization. However it also suggests that in order to limit the burden of malaria morbidity and mortality on pregnant women, it is necessary that treatment be administered in cases of asymptomatic malaria infection to the affected subjects in order to reduce the epidemiological impact.

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