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Research Article

Some Immunological Profiles in Women Practising DMPA Contraception in Malaria Endemic Area of Nnewi and Environs

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Abstract

Many women are now enlisting in family planning clinics in a bid to spacing their children, but there are attendant complications often associated with the use. This study evaluated the effects of injectable Depot Medroxyprogesterone acetate (DMPA) contraceptive on some immunological profile in females attending family planning clinic in NAUTH, Nnewi. Fifty female DMPA contraceptive users were recruited into this study and were further subdivided into malaria positive and malaria negative groups before and three months after administration of DMPA. The study was a longitudinal cohort design and simple random sampling technique was used in the recruitment of subjects. Blood samples were collected from the subjects before and 3 months after administration of DMPA, Immunological profile (IgG (µg/ml), IgM (ng/ml) and IFN-γ (pg/ml) were done using Enzyme linked immunosorbent assay. Qualitative and quantitative determination of *P. falciparium* antigen parasite were done by rapid test device and Giemsa stained thick blood film for microscopic determination respectively. CD4+ T-cells (cell/µl) were analyzed by flow cytometry. The results showed that the mean±SD serum level of IgM (ng/ml) was significantly decreased 3 months after administration of DMPA (213.45±75.64) compared with value before DMPA administration (363.06±5.63) with or without malaria parasite infection (p=0.000).However, there was no significant difference in mean±SD serum levels of IgG, CD4+ T-cell, IFN-γ before and 3 months after administration of DMPA (p>0.05) there was no significant difference in mean±SD serum levels of IgG, IgM, IFN-γ, CD4+ T-cells between malaria positive and malaria negative subjects before and 3 months after administration of DMPA (p>0.05). Thus, the finding of the study suggests that DMPA did affect the IgM response, but no significant changes were observed in immunological parameters studied when malaria is factored.

Keywords: Medroxyprogesterone Acetate; *P. falciparium*; Glucocorticoids

1. Introduction

1.1 Background

The current prevalence rate of contraceptive use in Nigeria is approximately 11%-15% [1]. This rate is very low in spite of the high rate of sexual activity and widespread awareness of the various contraceptive methods among Nigerian youths. As a result, there are many unintended pregnancies and illegal abortions contributing to a high maternal mortality ratio, which seems to indicate a large gap for contraceptive use [2]. Various factors that contribute to the low prevalence of modern contraceptive use in Nigeria have been identified [3]. The most common factor being the myth about the side effects of modern contraceptives [4].

However, Nigeria lacks the political will to provide family planning programs on a much larger scale, using community-based approaches and communication programs, to help change the myth about the side effects of modern contraceptives. The different modern contraceptives were largely acceptable to the Nigerian women, although the level of acceptability varied across the states, with the lowest levels of acceptability recorded in the two northern states of Kano and Adamawa [3]. Reason being affordability, availability, reliability and religion [4].

DMPA can prevent pregnancy through several mechanisms. Depot medroxyprogesterone acetate (DMPA) primarily acts by inhibition of gonadotropin secretion, thereby inhibiting follicular maturation and ovulation [5]. The inhibition of ovarian function results in hypoestrogenic state, which inhibits endometrial proliferation and renders the endometrium less receptive to implantation. DMPA also causes changes in cervical mucus (thicker and less permeable to sperm) and tubal motility (reduced ciliary action) that are unfavorable to sperm migration, thus inhibiting fertilization. DMPA is a highly effective contraceptive. The unintended pregnancy rate for the first year of perfect use of DMPA has been estimated at 0.2% [6].

To date, many studies have demonstrated a correlation between the use of hormonal contraception and increased risk of HIV-I infection [7]. One of the studies in Kenya showed that women who used DMPA were twice as likely to acquire HIV-I compared with no contraception [8]. Another recent report from demographic and Health surveys in four African countries confirmed higher HIV-I Sero-prevalence in DMPA users and estimated that 6% of new HIV-I cases are attributable to DMPA use [9]. However, non-human primate studies showed that administration of DMPA enhances the risk of HIV acquisition by more than 7-fold and significantly increases viral levels in the acute phase of infection [10].

Medroxyprogesterone, suppresses IL-1, IL-2 and IL-6 release by human lymphocytes to a similar extent as classic glucocorticoids dexamethasone and hydrocortisone [11]. Liganded GR-mediated repression of IL-2 gene expression is thought to be due to the direct interaction of GR with transcriptional enhancers such as activators protein-1 and

nuclear factor-KB [12]. Thus, by binding to the GR, medroxy progesterone, the active component of many contraceptive preparations may exert significantly stronger immunosuppressive activity.

A number of naturally occurring species of steroid hormones are able to qualitatively and quantitatively influence the production of lymphokines by activated T-cells *in vitro* [13]. This process may also probably occur *in vivo* [13]. For instance, lymphocytes are known to have receptors for a wide variety of hormones, including corticosteroids, insulin, catecholamines, growth hormone and met-enkephalin [14].

It seems apparent that the brain-pituitary-reproductive-axis and the brain-thymus-lymphoid-axis are linked by an array of internal mechanisms of communication that use similar signals (neurotransmitters, peptides, growth factors, hormones) acting on similar recognition targets [15]. Activation of the immune system has profound effects on endocrine function which are mediated by cytokines, including tumor necrosis factor-alpha (TNF-α) [16] and vice versa.

Thus, glucocorticoids (Gc), influences immune and inflammatory responses through their suppressive actions [16]. Oestrogen studies in animal models have demonstrated inhibition of platelet aggregation [16]. Oestrogen also suppresses cellular immunity, but stimulates humoral immunity, that is, deficiency promotes cellular (Th1-type) immunity while progesterone stimulates a switch from TH1 to Th2 type immune responses. Testosterone is also known to suppress both cellular and humoral immune responses [16]. The present study is designed to evaluate the immunoglobulin responses in the female following contraceptive administration.

2. Materials and methods

2.1 Subjects

This is a longitudinal study involving 75 first time DMPA users (aged 20-49 years) who were systematically recruited from family planning clinic NAUTH, Nnewi. Although 75 clients that met the selection criteria were enrolled into the study, only 50 started and completed the study giving a response rate of 66.7%. They were initially screened for pregnancy as well as malaria parasite infection using rapid test kits (Acon laboratories) and confirmatory methods (Giemsa stained thick smear). They were categorized into malaria positive and malaria negative subjects. Thereafter, 6mls of blood samples were collected before the administration of DMPA and 3 months after DMPA administration. Due to the fact that NAUTH family planning clinic employs “Quick start” method as their contraceptive method (i.e. they administer DMPA on the first day the subject is seen for contraception rather than waiting until the beginning of her next menstrual cycle as long as pregnancy is ruled out), the users menstrual cycle is not noted in this study.

The age, parity, history of malaria and contraceptive use were recorded using a standard questionnaire. Written informed consent was sought and obtained from all participants, and the study was approved by the Ethics Review Committee of NAUTH, Nnewi.

3. Methodology

Immunological profile (IgG, IgM and IFN-γ) were done using Enzyme linked immunosorbent assay. Qualitative and quantitative determination of *P. falciparium* antigen parasite were done by a rapid test device and Giemsa stained thick blood film for microscopic determination respectively. CD4+ T cells were analyzed by flow cytometry.

3.1 Statistical analysis

Statistical package for social science (SPSS) version 21 was used for the analysis of the data obtained. Values were presented as mean (±SD). Differences in continuous data were compared using Student's t-test and one way analysis of variance ANOVA) followed by the post hoc test (LCD). Correlation of parasite density with immunological markers and hormone were determined by Pearson's correlation coefficient analysis. All results were considered significant at ($p<0.05$).

4. Results

4.1 Mean (±SD) Age, BMI and Malaria parasite density before and after DMPA injection

The mean (±SD**)** age of female practicing DMPA contraceptive was 35±4.0 years while BMI before administration of DMPA (23.4±3.3) and after administration (25.6±3.5) were similar. Similarly, malaria parasitaemia did not differ before administration of DMPA (406.5±343.3) and after administration (473.6±376.9) (P>0.05) (Table 1).

4.2 Serum levels of IgG (µg/ml), IgM (ng/ml), IFN-γ (pg/ml) and CD4+T (cell/µl) cell before and after DMPA Injection

The mean (±SD) serum levels of IgGb (µg/ml) in the female before administration of DMPA was (17.6±6.3), and after 3 months of DMPA use (20.8±5.74) (t=-1.963;p<0.055). Between group comparisons showed that serum level of IgG before and after DMPA administration were similar (Table 2).

Parameters	Before	After	t=test	P=value
Age (years)	35±4.0	35±4.0	-0.050	0.960
Weight(kg)	69.73±12.65	70.55±12.92	-0.320	0.750
BMI (kg/m²)	25.4±3.3	25.6 ± 3.5	-0.322	0.749
MD (µL)	406.5±343.3	473.6±376.9	-0.655	0.517

Table 1: The Mean (±SD) Age, Weight, BMI and Malaria density before and after administration of DMPA.

Parameters	Pre injection N=50	3months DMPA N=50	t- test	p-value
IgG (µg/ml)	17.65±6.34	20.38±5.74	-1.963	0.055
IgM (ng/ml)	363.06±128.85	213.45±75.64	7.771	0.000*
IFN-γ (pg/ml)	70.52±23.00	70.78±27.98	-0.050	0.960
CD4+ (cell/µl)	839±249	822±234	0.354	0.0725

Table 2: Mean (±SD) Serum Levels: IgG(µg/ml), IgM(ng/ml), IFN-γ(pg/ml), and mean(±SD) blood level of CD4+ T Cell before and 3 months after administration of DMPA.

4.3 Serum levels of immunological parameters (IgG, IgM, IFN-γ and CD4+T Cell) before and after DMPA in subjects infected with or without malaria

The serum levels of IgG (µg/ml) (16.68±5.55), and IgM (ng/ml) (344.79 ±112.22), IF-γ(72.78±23.10) and CD4+T Cell (826.65±227.76) in malaria parasitaemic females before administration of DMPA was not significantly different compared to the IgG (19.92±7.60), IgM (405.79±157.25), IFN-γ (65.25±22.74) and CD4+T Cell (817.17±244.88) in non-malaria parasitemic female before administration of DMPA (P>0.05).

	Parameters	**MP Negative (N=15)**	**MP Positive (N=35)**	**t-test**	**p-value**
Before DMPA	IgG	19.92±7.60	16.68±5.55	1.691	0.097
	IgM	405.79±157.25	344.79±112.22	1.557	0.126
	IFN-γ	65.25±22.74	72.78±23.10	-1.061	0.294
	CD4+T Cell	817.17±244.88	826.65±227.76	-0.142	0.888
After DMPA	IgG	18.36±6.81	21.24±9.41	-1.068	0.291
	IgM	203.54±85.46	217.69±71.95	-0.602	0.550
	IFN-γ	73.26±18.27	69.72±31.41	0.406	0.686
	CD4+T Cell	640.33±251.66	610.57±213.41	0.428	0.670

Table 3: Serum levels of IgG, IgM, IFN-γ and CD4+T Cell before and after DMPA administration in subjects infected with and without malaria.

Similarly, there were no significant differences in serum levels of IgG (21.24 ±9.41), IgM(217.69 ± 71.95), IFN-γ(69.72 ± 31.41) and CD4+T Cell (610.57 ± 213.41) in malaria parasitaemic females after DMPA administration compared to the serum levels of IgG (18.36 ± 6.81), IgM (203.54 ± 85.46), IFN-γ (73.26 ± 18.27), CD4+T Cell (640.33 ± 251.66) in non-malaria parasitemic females after DMPA administration (P>0.05) (Table 3).

5. Discussion

In the present study, there was no significant difference in BMI before and after three months of DMPA administration. This finding is similar with previous reports by Ahmed (2015), who in their study of Babylonian women reported no statistically significant difference in weight after three months of DMPA use. The BMI of the female subjects in the present study was within the pre-obese range as defined by WHO [17] criteria. Obesity tends to reduce the efficacy of contraceptives because of their pharmacokinetics alterations [18]. A study in American women, showed that DMPA is a predictor of weight increase. Lopez *et al.* [19] showed that the mean weight gain was greater for DMPA users than users of IUD after a period of one, two and three years. In Ghananian women, a 5 years study showed a significant increase in weight of DMPA users compared with controls [20]. An increase in weight was also reported in Nigerian women who used DMPA when compared with IUD users over a period of 1 year.

Considering the findings in this present study about no gain in weight and those cited from other studies; it is clear that three months administration of DMPA has no effects on weight but long term use may have an effect.

There was no significant difference in parasitaemia before and after the DMPA administration in this present study. This may imply that DMPA users were not more susceptible to malaria parasitemia than non-users, since some of the immune index in malaria infected and non- malaria infected were similar. The absence of significant change in parasitaemia seen in this present study differs from those of Bray [21] in Gambia who reported that women who took progesterone contraceptive (ovral ^{R}) had an impaired immune response to malaria and enhanced parasitaemia. Additionally, Collins *et al.* [22] have reported that female Rhesus monkeys treated with such steroids maintained an increased cumulative malaria parasite load when compared with animal control. The discrepancies seen in the result of the present study and previous reports may reflect differences in the duration of use, concentration and population characteristics.

In this present study, two serum immonoglobulins IgM and IgG were measured in women using DMPA. Serum IgM concentration was reduced in women on DMPA over a period of 3 months. Previous studies by Lali *et al.* [23] and Wahda (2007) on women using DMPA contraceptive did not show any significant change in their studies which lasted between 1-12 months period. Additionally, Adekunle *et al.* [24] studied the effect of UniplantR on immunoglobulin concentration and reported no significant change in serum levels. Adekunle and co-workers used UniplantR which is a newer long acting 19 Nor progesterone derivative. The difference in findings between this

present study and that of Adekunle *et al.* could perhaps be due to the mode of administration of the contraceptive. While DMPA is administered intramusculary, UniplantR is inserted subcutaneously. The present study did not observe any significant change in serum IgG level in women using DAMP. But an increase in serum IgG levels was reported by Lali *et al.* (1996) and Wahda [25]. Lali *et al.* observed an increase in serum IgG concentration after 1 month of DAMP use, but no change in IgG level after a period of 3 months, while Wahda observed a significant increase in serum IgG levels after a period of 12 months. The higher serum levels of immunoglobulin in females may suggest a stimulating effect of female sex hormones or an inhibitory effect of testosterone upon this parameter. However, this proposed up- regulation and antibody switch by sex hormones such as estrogen was not observed in the present study and probably explains why no significant change was observed.

Furthermore, in this present study IFN-γ level was not altered in women after 3 months period of DMPA use. This is similar to the findings of Agarawal and Marshall [26] and Faas et al. [27] who showed that lymphocyte IFNγ production was not affected by synthetic hormones, but in contrast with the findings of Giron-Gonzalez et al. [28]. IFN-γ is secreted by the T lymphocytes and has been shown to be involved in the initial response in clearing of infective pathogens. Several invivo and invitro studies showed that neither progesterone, 17β-E2 nor testosterone altered IFN-γ production [29]. Combing various studies [30] concluded that there are no effects of sex hormones on lymphocyte IFN- γ production.

6. Conclusion

This study revealed no significant difference in level of some immunological profiles (IgG, $CD4^+$ T-cell and IFN-γ) before and after the administration of DMPA. However, a significant decrease was observed in the level of IgM three months after administration of DMPA than before the Similar immune response found in malarious and non malarious subjects showed that DMPA users are not more susceptible to malaria infection than non-users. No significant changes was observed in immunological parameters studied when malaria effect was factored.

7. Recommendation

Further long term studies are needed to definitively conclude that DMPA is associated with an improved immunological status.

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