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Serum immunoglobulins, total protein and albumin levels during Uniplant[R] use by Nigerian Women

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Summary

The effects of Uniplant[R] (a new, long-acting, 19-nor-progesterone derivative contraceptive) on serum immunoglobulins, albumin and total proteins were determined in Nigerian women during one year of use. Blood samples were collected prior to implant insertion and then at the third, sixth and twelfth months of use. All volunteers were in the reproductive age, healthy and had no contraindications to hormonal contraception. The mean levels of IgG ($\pm$ SD) increased from pre-insertion to the twelfth month. When compared with the pre-insertion level (1,393.93 $\pm$ 93.51 mg/dL), there are statistically significant increases in the mean values of IgG at three (1,457.19 $\pm$ 78.41 mg/dL, $p<0.05$), six (1,458.12 $\pm$ 65.26 mgd/L, $p<0.05$) and 12 months (1,499.56 $\pm$ 87.60 mg/dL, $p<0.001$). There were no statistically significant changes observed in the mean serum levels of IgA, IgM and total proteins during twelve months of implant use. These results indicate that while Uniplant does not seem to alter the levels of IgA, IgM, albumin and total proteins over a period of twelve months, it may induce significant increase in IgG levels. The raised mean serum levels of IgG may suggest an improved humoral immunity of Uniplant – a change that is potentially beneficial.

Keywords: *Immunoglobulins, total proteins, progestins, Uniplant[R] subdermal implants.*

Résumé

les effets d' Uniplant[R] (un nouveau anticonceptionel à long effect et derivé du 19-non-progestérone) sur les sérums immunoglobulines et albumine dont les protéines ont été déterminés au cours d'un an d'utilisation par les nigérianes. Des prélèvements de sang ont été faits avant l'insertion de l'implant et s'en est suivi dans le troisième, sixième et douzième mois de l'utilisation. Toutes les volontaires étaient dans la fleur de l'age, en bonne santé, n'avaient aucune contre-indication de contraception hormonale. Les niveux moyens de IgG ($\pm$ SD) ont augumentés d'avant l'insertion jusqu'au douzième mois d'insertion. Il y a des augumentations statistiquement signifiantes dans les valeurs moyennes de IgG au troisième mois (1.457.19 $\pm$ 78,41 mg/dL, $P<0,05$), au sixième mois (1.458.12 $\pm$ 65,26 mg/dL, $p<0,05$) et au douzième mois (1,499,56 $\pm$ 87,60 mg/dL, $P<0,001$) comparativement au niveu de préinsertion (1,393,12 $\pm$ 93,51 mg/dL,). Aucun changement statistiquement significatif n'a été observé dans les niveaux de moyen de sérum d'IgA, d'IgM et la totalité de protéines pendant douze mois d'usage d'Uniplant[R] Ces résultats montrent que, quand bien même, il semble que l'Uniplant ne modifie pas les niveaux d'IgA, IgM, albumine et la totalité des protéines pendant une période de douze mois, il pourrait provoquer une augumentation significative des niveaux d'IgG. L'élévation dans les niveaux de la moyenne de sérum d'IgG peut suggerer une amélioration d'immunité homorale d'Uniplant – un changement qui est potentiellement bénéfique.

Introduction

The introduction of contraceptive implants represents a milestone in continued research effort on contraceptive delivery systems to ensure sustained release of steroids into tissues through biocompatible silicone elastomers at low, stable concentrations for years without compromising on contraceptive effectiveness [1]. In the last three decades, at least ten compounds have been tested as implantable contraceptives in over 5,000 women worldwide [2]. Nomegestrol acetate implant (Uniplant[R]) is a new, long-acting, 19-nor-progesterone derivative contraceptive which has recently been added to our contraceptive armamentarium at Ibadan, Nigeria as part of a multicentred introductory clinical trial [3]. Preliminary reports are assuring with regard to the safety of the long-term use of Uniplant in respect to carbohydrate metabolism, serum lipoproteins, hepatic function [3,4], androgens and sex hormone binding globulin levels [5].

The current widespread use of synthetic estrogens and progestins for contraceptive purposes provides an opportunity for assessing the influence of these steroids on various biochemical parameters of the female, specifically to identify any association between the metabolic and side-effects of the agents. Previous reports on the effects of combined oral contraceptives on serum immunoglobulin levels have been conflicting. While some workers reported an increase in one or more immunoglobulins [6,7], others have reported a decrease [8,9], and a few, no change [10]. Similarly, progestin-only preparations have been shown to have equivocal effects on serum immunoglobulin levels in users [11-13]. In a study of the effects of Norplant[R] (levonorgestrel acetate implant) on immunoglobulin levels in Nigerian women, serum levels of IgG showed statistically significant increases after one, three and twelve month of implant use [14]. However, there were no statistically significant changes observed in the serum levels of IgA, IgM, total proteins and albumin. The present study is the first attempt to assess the effects of Uniplant[R] on the serum immunoglobulin, albumin and total protein levels in indigenous Nigerian users.

Materials and Methods

Thirty consecutive healthy women seeking contraception for birth spacing and who after counselling, chose Uniplant subdermal implant, were recruited into the study. All subjects were recruited from the Family Planning Clinic, University College Hospital, Ibadan, Nigeria. They had not use any form of hormonal contraception for at least one year before recruitment and had no contraindication to its use. Those with a history of liver disease, jaundice or conditions that may affect the immune response such as infections were excluded. The study was approved by the Joint Hospital and University Ethical Committee

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and informed written consent was obtained from all participants.

The implants, which were supplied by the South to South Cooperation in Reproductive Health, Salvador, Brazil, were made from medical grade dimethylpolysiloxane (silastic) tubing (Dow Coning, Midland, MI, USA). Segments measuring 39 mm in total length (35 mm of filled length) and 2.4 mm in diameter were used to make the implant. The segments of Silastic tubing were filled with 55 mg (10%) of crystalline, finely ground nomegestrol acetate (3,20-oxo-6-methyl-17-alpha-acetoxy-19-norpregna-4,6-diene; Theramex, France) and sealed at both ends with Silastic medical adhesive, type A, as previously described [3,4]. Implants were then steam sterilized.

Each subject was seen between 8.00 a.m. and 9.00 a.m. on the day of insertion and following intracutaneous local anaesthesia with 1% xylocaine, one capsule was inserted subcutaneously in the medial part of the left upper arm. Before the procedure, 10 ml of fasting venous blood was collected from the antecubital vein of either arm into an anti-coagulant-free tube. Subsequent samples were obtained at about the same time of the day from those who continued to use the implant at the end of the third, sixth and twelfth month following insertion. After clot retraction, the bottle was spun at 3,000 rpm for five minutes. The sera were separated and stored at –20°C until analyzed at the end of the fifteenth month following the last insertion.

The immunoglobulin levels in the sera were estimated by the modified single radial immunodifusion techniques of Fahey and Mckeley [15] as modified by Salimonu et al. [16]. Total protein levels were assayed by the Biuret method [17] and albumin by the Bromocresol purple method [18]. The interassay and intraassay errors were less than 10% by the immunodifusion technique of Salimonu et al. [16], and less than 8.7% by the Biuret method [17]. Each patient acted as her own control.

Statistical analysis was carried out using the Microsoft Excel statistical package. Descriptive statistics were obtained for immunoglobulin IgG, IgA, IgM, total proteins and albumin levels. The mean levels at 3, 6 and 12 months were compared with the pre-insertion levels using the paired "t" test to determine the level of significance of any observed differences.

Results

Twenty-seven of the thirty women recruited completed the study and their results were analyzed. Their mean age was 31.19 ± 6.01 years and mean parity was 3.1 ± 1.60. The values of IgG of the study group over a twelve-month period are shown in Figure 1. The mean levels of IgG (± SD) increased from pre-insertion to the twelfth month. When compared with the pre-insertion level (1,393.93 ± 93.51 mg/dL), there were statistically significant increases in the mean values of IgG at three (1,457.19 ± 78.41 mg/dL, $P<0.05$), six (1,458.12 ± 65.26 mgd/L, $P<0.05$) and 12 months (1,499.56 ± 87.60 mg/dL, $P<0.001$). The mean (± SD) value of IgA at insertion was 136.26 ± 30.40 mg/dL, while at three, six and twelfth months, they were 129.31 ± 34.45 mg/dL, 147.84 ± 39.33 mg/dL and 137.30 ± 40.35 mg/dL, respectively (Figure 2). There was a slight but insignificant increase at six months of implant use but it fell at twelve months. The mean values of IgM decreased slightly from the pre-insertion level to the twelfth month (Figure 3). These changes were

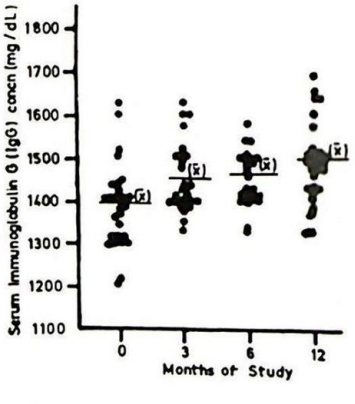

Fig. 1: Serum immunoglobulin G levels of the study group over 12 months.

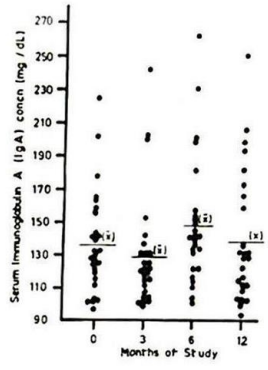

Fig. 2: Serum immunoglobulin A levels of the study group over 12 months

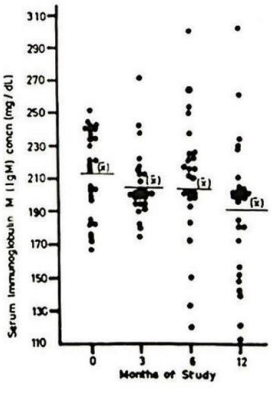

Fig. 3: Serum immunoglobulin M levels of the study group over 12 months

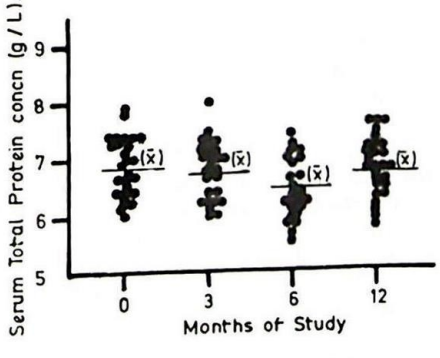

Fig. 4: Serum total protein levels of the study group over 12 months

not statistically significant. Figure 4 shows that the mean values of serum total proteins decreased slightly at the third and sixth months of implant use but rose again at the end of one year. The mean levels of serum albumin increased, albeit insignificantly from pre-insertion to the twelfth month of implant use (Figure 5). Again, these changes were not statistically signifi

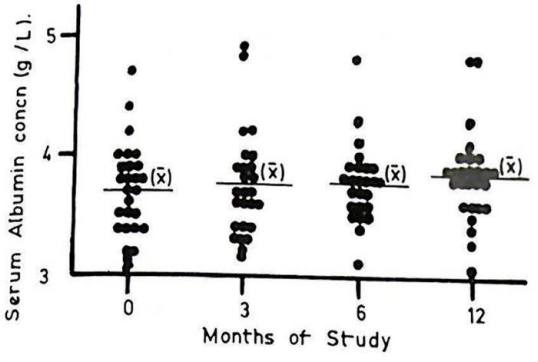

Fig. 5: Serum albumin levels of the study group over 12 months

cant. The mean levels of IgG, IgA, IgM, serum total proteins and serum albumin are presented in Table 1. The only significant changes, as earlier described, were in the mean values of IgG at three, six and twelve months of Uniplant[R] use when compared to the pre-insertion level.

Although, some workers in Egypt [10,21] did not find any significant changes in the mean serum levels of IgG, IgA and IgM in Egyptian women on Norplant[R] subdermal implants, a study in Nigerian users demonstrated an increase in IgG levels [14]. The patterns of change in the Nigerian Norplant[R] study was almost similar to that observed in this study. In the Norplant[R] study [14], the rise was early and already significantly noticeable at one and three months of implant use. However, it fell at the twelfth month. Lali et al. [22], while studying the effects of Depot Medroxyprogesterone acetate (DMPA) on immunoglobulins, total proteins and albumin levels in Indian women, observed an increase in the levels of IgG in the first and third months of use. The changes at one month were statistically significant when compared with the pre-injection levels.

These findings may suggest a selective stimulation of IgG synthesis associated with endogenous progestin use. The mechanism for this increase in IgG levels remains unclear. Liver stimulation by the progestin has been proposed as a possible pathway [14] and indeed, some workers had concluded that the changes in the plasma proteins were probably caused by the liver [8]. However, Barbosa et al. [4] had earlier demonstrated that there were no significant changes in the hepatic function in women on Uniplant as only minor and transient changes in the liver enzymes and bilirubin were observed. An evidence in support of the possibility of immune enhancing effects of steroid hormones is suggested by the findings of a recent in-vivo study [23] in the United Kingdom in which the potential of steroid to act as immuno-stimulatory factors was demonstrated.

Table 1: Changes in serum Immunoglobulins, total proteins and albumin levels (mean + SD) in Uniplant users

Month	IgG (mg/dL)	IgA (mg/dL)	IgM (mg/dL)	Total Protein (g/L)	Albumin (g/L)
Month 0	1393.93 ± 93.51	136.26 ± 30.40	213.04 ± 27.77	6.83 ± 0.52	3.70 ± 0.39
Month 3	1457.19 ± 78.41*	129.31 ± 34.45	205.23 ± 20.50	6.78 ± 0.52	3.75 ± 0.43
Month 6	1458.12 ± 65.26*	147.84 ± 39.33	208.76 ± 39.01	6.49 ± 0.53	3.78 ± 0.32
Month 12	1499.56 ± 87.60**	137.30 ± 40.35	190.56 ± 41.89	6.79 ± 0.51	3.85 ± 0.38

** $P < 0.05$ when compared with pre-insertion level.*
*** $p < 0.001$ when compared with pre-insertion level.*

Discussion

Specific effects of estrogens and estrogen-progestin combinations on the levels of a number of plasma proteins have been reported [19,20]. Our results show that there were no significant changes observed in the serum levels of immunoglobulins IgA and IgM, and that the total protein and albumin levels showed virtually no fluctuations during one year of Uniplant use. The mean levels of IgG at three, six and twelve months showed a statistically significant increase when compared with the pre-insertion level. Similarly, Horne et al. [6] and Chandra [7] found such increases in the IgG and IgA levels in women on the combined oral contraceptive pills. Gleichmann *et al.* [8] observed that oral contraception by estrogen-progestin preparations significantly changed the concentrations of twelve out of sixteen immunologically assayed serum proteins. The majority of the alterations became apparent after one month of oral contraception and were little affected by further treatment. Estrogen seemed to be responsible for changing the concentration of albumin and immunoglobulin A, among others, while prealbumin was influenced by certain progestins.

Infections, particularly malaria (which is endemic in tropical Africa) and others such as bacterial result in increases of IgG levels [21]. A previous study had demonstrated a seasonal fluctuation in serum concentrations of immunoglobulins in Nigerians [24]. While lower levels of immunoglobulins were observed in the dry season, increased concentrations of serum IgG, IgM and IgA were detected in the rainy season, which also corresponds with the peak malaria period. These findings of differences in concentrations between seasons have been attributed to a wider range and severity of antigenic challenges to which the population of developing countries is exposed, especially in the rainy season. Recently, elevation of IgG levels was observed in Nigerians with urinary schistosomiasis [25]. To eliminate the effects of these infections and such other conditions that could affect the immune responses, apparently healthy subjects were investigated throughout this study. Uniplant did not seem to have any effect on serum proteins and albumin. Similarly, Laurell *et al.* [26] had earlier indicated that progestogens in doses used for contraceptive purposes after long term treatment induced no changes in the protein metabolism as result in altered levels of plasma proteins.

We conclude from our findings that Uniplant does not seem to alter the serum levels of immunoglobulins IgM, IgA, albumin and total proteins in Nigerian users. It however, induces significant changes in IgG levels of Nigerian women by the third, sixth and twelfth month after insertion. The raised mean serum levels of IgG may suggest an improved humoral immunity of the contraceptive method – a change that is potentially beneficial. Future studies will aim at characterizing the antibody specificity of these raised immunoglobulin G levels observed during Uniplant use.

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