

Effect of depot-medroxyprogesterone acetate injections on serum immunoglobulins and complements level cohort study

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ABSTRACT

Background: Depot medroxyprogesterone acetate (Depo-Provera) is a highly effective, nondaily hormonal contraceptive option that has been available in the worldwide for ٤٠ years and provides a very high level of contraceptive efficacy. Benefits and risks of hormonal therapy are often under scrutiny, however, long-term clinical experience has established the safety of this long-acting contraceptive in that it does not increase the risk of cardiovascular events, breast cancer , other gynecologic malignancy, or postmenopausal fracture.

Aim: To evaluate the effect of depot medroxyprogesterone acetate injections on the serum immunoglobulin (IgG, IgM, IgA), and complements (C^٣,C^٤).

Study design: Prospective cohort study.

Study period: ١st January ٢٠٠٦ to ٣١st March ٢٠٠٧.

Subjects & Methods: Thirty healthy married women, their age ranged between ٢٠-٣٥ years, who were attending Al-Batool and Al-Khansa Family Planning Centers in Mosul/ Iraq and started (for the first time) to use depot medroxyprogesterone acetate (DMPA) injection. Each injection contains ١٥٠ mg medroxyprogesterone acetate. These women called DMPA users group, were compared to another thirty healthy married women who did not use any hormonal contraceptives and were drawn from the same population and matched for age, height and weight with the DMPA users group, and they were considered to be the non users group. Both groups were followed for one year, during which blood samples were obtained from both groups, before starting to use DMPA, after ٦ months and then after ١٢ months. The sera obtained from the blood samples were used for the estimation of the biochemical studied parameters using commercial kits.

Results: Serum IgG was significantly elevated after ١٢ months ($p = ٠.٠٢٨$) among DMPA users in comparison to non users but non significant increase according to the duration of use. While there was a non significant changes in the serum IgM, IgA, C^٣ and C^٤ level after ٦ and ١٢ months in the DMPA users in comparison with non users and in relation to the duration of use. DMPA caused a non significant changes in the total serum protein and serum albumin levels.

Conclusion: The significant raise of serum levels of IgG may be indicative of improved humeral immunity in DMPA Iraqi users, a change that is potentially beneficial.

Keywords: immunoglobulin, complements, depot-medroxyprogesterone

الخلاصة

الخلفية: حقن مخزن ميدروكسي بروجيسترون أسيتيد هي من موانع الحمل الهرمونية عالية الكفاءة والتي لاتعطى يوميا ومتوفرة في العالم منذ أربعين عاما وتعطى منع حمل عالي الكفاءة. فوائد و مخاطر العلاج بالهرمونات عادة تحت الفحص والتدقيق و مع هذا فان التجارب السريرية أثبتت أن موانع الحمل طويلة المفعول مأمونة من ناحية أنها لاتزيد من خطورة أمراض القلب الشريانية، سرطان الثدي والسرطانات النسائية الأخرى ولا الكسور بعد سن اليأس.

الهدف: لتقييم تأثير حقن مخزن ميدروكسي بروجيستيرون أسيتيد على مستوى مصل الدم للكلوبيولينات المناعية نوع (IgG)G, (IgM)M, (IgA)A و المتممات نوع ٣ (C٣) و نوع ٤ (C٤) لدى مستعملات حقن مخزن ميدروكسي بروجيستيرون أسيتيد.

تصميم الدراسة: دراسة جماعة. **فترة الدراسة:** الأول من كانون الثاني ٢٠٠٦ الى الحادي والثلاثين من اذار ٢٠٠٧ **الطرق المتبعة والأشخاص:** تم أخذ مجموع ٣٠ امرأة سليمة تتراوح أعمارهن بين (٢٠ - ٣٥) سنة ممن كن يراجعن مركزي تنظيم الأسرة في مستشفى البتول والخنساء في الموصل وممن بدأن لأول مرة بأستعمال حقن مخزن ميدروكسي بروجيستيرون أسيتيد كمانع حمل. كل حقنة تحتوي على ١٥٠ ملي غرام ميدروكسي بروجيستيرون أسيتيد. هؤلاء النساء سمين المجموعة المستعملة لمخزن ميدروكسي بروجيستيرون أسيتيد وقد تم مقارنتهن مع ٣٠ امرأة سليمة أخرى من نفس الفئة العمرية والطول والوزن ممن لا يستعملن موانع الحمل الهرمونية وتم اعتبارهن "كمجموعة ضبط أو سيطره". كلا المجموعتين تمت متابعتهم لمدة سنة واحدة وخلالها تم سحب دم من كل امرأة من كلا المجموعتين وعلى ثلاث فترات وهي قبل البدء بالمتابعة وأستعمال حقن مخزن ميدروكسي بروجيستيرون أسيتيد وبعد ٦ اشهر ثم بعد ١٢ شهرا. تم استخدام عينات مصل الدم المأخوذ من نماذج الدم لقياس الفحوصات الكيمياوية بأستعمال العدد اليدوية التجاربه.

النتائج: أن حقن مخزن ميدروكسي بروجيستيرون أسيتيد سببت زيادة معنوية في مستوى مصل الدم للكلوبيولين المناعي نوع G (IgG) بعد ١٢ شهر لدى مستعملات حقن مخزن ميدروكسي بروجيستيرون أسيتيد مقارنة بغير المستعملات ولكن سببت تغيير غير معنوي لدى مستعملات حقن مخزن ميدروكسي بروجيستيرون أسيتيد مقارنة بمدة الاستعمال. بينما سببت تغيير غير معنوي في مستوى مصل الدم للكلوبيولينات المناعية نوع M (IgM) و (IgA)A و المتممات نوع ٣ (C٣) و نوع ٤ (C٤) لدى مستعملات حقن مخزن ميدروكسي بروجيستيرون أسيتيد مقارنة بغير المستعملات ومدة الاستعمال. حقن مخزن ميدروكسي بروجيستيرون أسيتيد سببت تغيير غير معنوي في مستوى البروتين الكلي ومستوى البومين في مصل الدم.

الأستنتاج: أن الأرتفاع المعنوي في معدل الكلوبيولينات المناعية نوع G (IgG) قد يدل على تحسن المناعة لدى مستعملات مخزن ميدروكسي بروجيستيرون أسيتيد العراقيات، هذا التغيير قد يدل على فائدة.

Depot medroxyprogesterone acetate is a long acting progesterone provided as paranteral contraceptive. It provides a very high level of contraceptive efficacy and it is effective as combined oral contraceptive pills (COCPs). In addition, DMPA injections may be particularly good choice for women in whom COCPs are either contraindicated or may cause additional health concerns.^١ It is usually given within the first ٥ days of the menstrual cycle, by intramuscular injection as long acting preparations in a dose of ١٥٠ mg to provide contraception for up to ٣ months period.^٢

Episodes of irregular, unpredictable spotting and/or break through bleeding within the first year of using DMPA injections, are most frequently encountered side effects. Long term use of DMPA injections may cause a reduction of menstrual blood loss, decrease risk of endometrial cancer and suppress the endogenous estrogen secretion; which lead to: reversible reduction in the bone density and changes in plasma lipids which are associated

with increased risk of atherosclerosis^٣. Since atherosclerosis may in part, be an inflammatory disease,^٤ thus circulating factors that are related to inflammation may be predictors of cardiovascular diseases in general population.^٥

The complement system and immunoglobulins are the main components of humeral immunity. The activation of complement is known to be involved in a number of forms of cardiovascular disease, such as exacerbation of myocardial defect following ischemic injury by complement activation^٦ and may involved in the generation of spontaneous atherosclerotic lesions.^٧ The third complement component (C٣), is a cytokine acute phase protein, and is produced by activated macrophages^٨, liver and adipose tissue^٩. C٣ and its fragments are recognized regulators of the humeral immune response and B- cell proliferation.^{١٠}

Previous studies have clearly shown that both susceptibility and immune responses in the female genital tract are regulated by sex

hormones^{١١,١٢}. In a rat model, it was previously shown that the stage of the estrous cycle and treatment with sex hormones, specifically estradiol and progesterone, influence both the inductive and effector arm of the immune system in the genital tract^{١٣}. Antigen presentation, immunoglobulin A (IgA) and IgG levels, IgA transport, and presence of immune cells are all under hormonal regulation^{١٤,١٥}. More recently these studies have been extended to cover the reproductive tract of women, demonstrating that similar regulation of immune responses exists in women during the menstrual cycle.^{١٦}

It was found that the in vivo administration of medroxyprogesterone acetate (MPA) to mice, ٧٠ or ٩٠ days before immunization with sheep red blood, significantly enhanced antibody responses, without affecting delayed hypersensitivity. Also it was found that low doses of MPA significantly enhanced the in vitro production of IgG antibodies, and because MPA did not increase the number of immunoglobulin-secreting cells, it was suggested that MPA enhanced the capacity of individual cells to produce specific immunoglobulin.^{١٧}

The aim of this study was to evaluate the effects of DMPA usage for one year on serum immunoglobulins (IgG, IgM, IgA), and complements (C_٣, C_٤), and total serum protein and serum Albumin.

Subjects, Materials & Methods

This study included ٣٠ apparently healthy women who were attending Al-Batool Family Planning Centers in Mosul/ Iraq. The following inclusion criteria were put: The age ranges between ٢٠-٣٥ years old. Body Mass Index (BMI) for each woman should be < ٢٥ (BMI = weight (kg)/height (m^٢)).^{١٨} Each woman should be: Married, not pregnant, not lactating but were fertile at the time of study, having regular menstrual cycle, apparently healthy with hemoglobin, not less than ١٠.٥ gm/dl, not be a hormonal contraceptives users before, or using any medications during the period of the study, neither having history of allergy nor any disease that interfere with the immune system such as systemic lupus erythematosus, tuberculosis, rheumatoid arthritis and neither smoker nor alcoholic. They were just started to

receive (for the first time) DMPA injection called "Depo-Provera" of Pharmacia NV/SA Puurs-Belgium. Each injection contains ١٥٠ mg medroxyprogesterone acetate. These women called DMPA users group. The non users group included another ٣٠ apparently healthy women who have the same inclusion criteria as the DMPA users group except that they were not using any hormonal contraceptives, instead, they used either a barrier method or mechanical methods and they were volunteered for comparison. The two groups were followed by taking a detailed information which includes age in years, blood pressure (mmHg), body weight (Kg), height (cm), number of children, parity, gravity, menstrual cycle, disease state (history or family history of hypertension, diabetes mellitus), if using other drugs, about smoking or consumption of alcohol and type of contraceptives used before. About ١٠ ml of venous blood were withdrawn, using a disposable syringe at about ٨.٥-١٠ am from the DMPA injections users group at the beginning before they start taking the injection, after ٦ months, then after ١٢ months of use, and from the non contraceptive users group using the same schedule. The blood allowed to clot in a plain tube at room temperature and then the serum was separated by centrifugation at ٣٠٠٠ rpm for ١٠ minutes and then kept frozen at - ٢٠ °C to be analyzed for measuring serum level of:

- ١- Serum immunoglobulins (IgG, IgM, IgA) and serum complements (C_٣ and C_٤) which were measured by single radial immunodiffusion (RID) method using Kallestad Endoplate Single Radial Immunodiffusion Test Kit (Sanofi Diagnostics, France). The method of RID to quantitate Ig depends on the relationship between antigen concentration and the diameter of the precipitin ring formed when the antigen diffuses from a cylindrical well into an antibody containing gel. The endpoint is reached when all of the antigen has reacted with antibody.^{١٩}
- ٢- Total Serum Protein: was measured by Biuret Method,^{٢٠} using a Syrbio kit of total protein (Paris-France).
- ٣- Serum Albumin: was measured by Bromocresol Green Method.^{٢١} using Syrbio kit of albumin (Paris-France).

Standard statistical methods were used to determine the mean, standard deviation (SD) and the range. Paired t-test was used to compare the results of various biochemical parameters among DMPA users and non-users. Linear regression analysis (Pearson correlation coefficient R^2) was performed for finding the degree of association between different parameters. ANOVA Test (analysis of variance) was used to identify the variation in the different variables in relation to the duration of DMPA users group. While Duncan's test was used to identify groups responsible for statistical difference through comparison. All values quoted as the mean \pm SD and a P-value of < 0.05 was considered to be statistically significant.

Results

The (mean \pm SD) age of the DMPA users was 28.26 ± 4.14 years, while the (mean \pm SD) age of the non users was 27.40 ± 4.71 years. There was no significant difference between mean \pm SD height of the DMPA users (157.07 ± 3.32 cm.), and the mean \pm SD height of the non users (159.30 ± 3.20 cm.).

Table 1 demonstrates that there was a significant increased in the serum IgG after 12 months in the DMPA users in comparison with the non users. There was no significant difference in the mean serum IgG of the DMPA users and non users at the baseline time (0 month).

ANOVA analysis among the DMPA users group indicated a non significant increase in

the mean serum IgG according to the duration of DMPA injections use (Table 1).

Table 1: Comparison between mean serum IgG level of DMPA users

Period of use (Months)	(Mean \pm SD) Serum IgG (mg/dl)	
	DMPA Users (n=30)	Non Users (n=30)
0 Month	2027.2 ± 499.4 a	2300 ± 300
6 Months	2649.0 ± 528.1 a	2027 ± 400
12 Months	2808.3 ± 314.6 b	2022 ± 300

- (a , b) different letters (vertically), means significant difference.

mean C^{γ} of the DMPA users at the baseline time.

There were a non significant decrease in the total serum protein levels among DMPA users in comparison with the non users after 6 months but a significant decrease after 12 months was noted while a non significant changes in the mean serum albumin among DMPA users in comparison to the non users after 6 and 12 months, and a non significant difference in the mean total serum protein and the mean serum albumin of the DMPA users and the non users at the baseline time.

ANOVA analysis of the DMPA users group also indicated a non significant variation in the mean serum IgM, IgA, C^{γ} , C^{ξ} , total serum protein, mean serum albumin of the DMPA users in relation to the duration of usage.

Discussion

been determined that female sex hormones, especially estrogen and progesterone, have a

The difference between male and female immune response are well documented. It has

distinct effect on immune function¹⁷. The exact mechanism for this difference between men and women, however, is not clearly understood. Apparently both humeral and cellular immune activities are more aggressive in women than in men¹⁸.

The present study illustrates that there is a significant increase in the mean serum IgG level after 12 months of DMPA usage but a non significant variation in relation to the duration of use, while, non significance difference in IgM or IgA levels were observed. Although there was a significant decrease in total serum protein after 12 months of DMPA injections usage in comparison to non users, but no significance difference in the mean serum albumin level and a non significant variation in relation to the duration of use of both. This study is in agreement with the study done by Lali, *et al.*,¹⁵ to determine the effect of DMPA as contraceptive on the levels of serum immunoglobulins, total protein and albumin in Indian users and found that levels of total protein and albumin remained unchanged. No significant changes were observed in the levels of IgA and IgM, while IgG levels were increased in the first and third month after DMPA injection, and the increase in the first month was statistically significant ($P=0.019$). This was indicative of improved humeral immunity in Indian DMPA users.

While the study done by Fajumi¹⁹ found that treatment with DMPA injection produced increased serum levels of albumin, alpha 1-acid glycoprotein, alpha 2-macroglobulin, haptoglobin IgG, reduced levels of alpha 1-antitrypsin, transferrin, C₃, C₄ but no change in serum IgA, IgM, C-reactive protein and ceruloplasmin. The significant alterations were observed in serum proteins that are notably synthesized by the liver, an observation consistent with influences which gonadal hormones exert on the metabolic activities of this organ.

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

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during one year of use. Their 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 humeral immunity of Uniplant—a change that is potentially beneficial^{16, 20}.

Limited data were available from the literatures concerning the effect of DMPA on serum complement level. The data obtained from this study demonstrated that DMPA usage for one year caused a non significant changes in serum C₃ and C₄ level in the DMPA users in comparison with the non users after 6 and 12 months, and a non significant variation in relation to the duration of use. This study is in contrast with the work of Yilmazer *et al.*,²¹ who found that C₃ level is significantly increase in healthy post menopausal women using oral ethinyl estradiol (EE) + medroxyprogesterone acetate (MPA) than in similar women using transdermal 17β estradiol + MPA and those not on hormonal replacement therapy (HRT). In addition, serum C₄ level also increase in women receiving EE + MPA than untreated women.

The association of Ig with cardiovascular diseases remains the subject of debate. It has been proposed that serum IgA, IgG and IgE may be associated with cardiovascular diseases in dyslipidemic men²² but others^{23, 24} have suggested that the increase in IgM and IgG level might be a consequence of the primary involvement of C₃ as there was no independent association of IgM and IgG with atherosclerosis and myocardial infarction. In contrast to this, in the present study there is significant negative correlation between C₃ and IgM level ($r = -0.737$, $p = 0.010$) and a lesser non significant negative correlation of C₃ with IgG, IgA level ($r = -0.598$, $p = 0.068$; $r = -0.600$, $p = 0.067$) respectively.

This study concluded that the use of DMPA injections as contraceptives by Iraqi women cause a significant raise of serum levels of IgG, a change that is potentially beneficial as may be indicative of improved humeral immunity.

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