Prolactin levels in South African women on injectable progestogen contraceptives

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Objective. To demonstrate the effect of depot medroxyprogesterone acetate (DMPA) and norethisterone oenanthate (NET EN) on basal prolactin levels with a view to investigating the role of progestogen-induced hyperprolactinaemia in the pathogenesis of galactorrhoea, amenorrhoea and possibly prolactinomas.

Design. Descriptive study.

Setting. Commercial Centre Family Planning Clinic and King Edward VIII Hospital, Durban, South Africa.

Subjects. Seventy-four women on injectable contraceptives comprised the study group. Thirty-nine of these women were on DMPA and 35 on NET EN. The control group comprised 62 women. Women with medical conditions or medications that affected prolactin secretion as well as lactating and pregnant women were excluded from the study. Blood samples were obtained by venepuncture under controlled, standard conditions. Serum prolactin levels were determined by enzyme-linked immunosorbent assay.

Results. The overall mean serum prolactin level in the study group was 266 μIU/ml. In the control group the mean serum prolactin level was 245 μIU/ml. There was no significant difference between the two groups ($P = 0.39$). The mean serum prolactin level among women on DMPA was 226 μIU/ml compared with 310 μIU/ml among those on NET EN. Basal prolactin levels were significantly increased in women on NET EN compared with the control group ($P = 0.03$). There was no significant difference in basal prolactin levels between the control group and women on DMPA ($P = 0.43$).

Conclusion. The use of NET EN was associated with a significant increase in serum prolactin levels, although they remained within the normal range. Consequently NET EN may cause chronic hyperstimulation of anterior pituitary lactotrophs and may therefore predispose users to the development of prolactinomas. The risk, however, is probably minimal.

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Injectable progestogen contraceptives are among the most popular forms of contraception. Depot medroxyprogesterone acetate (DMPA) is currently used by 10 million women worldwide.[1] In a recent study in the Western Cape, 70% of the women surveyed had used DMPA or norethisterone oenanthate (NET EN), making these the most widely used forms of hormonal contraception in South Africa.[2]

The influence of endogenous progesterone on prolactin (PRL) secretion is controversial and differs among species.[3] Several clinical effects associated with the use of injectable progestogens are suggestive of progestogen-induced hyperprolactinaemia. Firstly, DMPA and NET EN are known to cause galactorrhoea in a small group of women. This may be associated with normal or slightly raised PRL levels.[3] Secondly, DMPA and NET EN may have positive effects on lactation. While some studies have only shown a lack of adverse effect on lactation,[4,5] other studies have demonstrated a slight but significant increase in measured milk volumes and duration of lactation.[6,7] Discrepancies between these studies may be attributed to poorly defined samples, inconsistent measurements of lactation and variations in dose of injectable contraceptives. However, Chaudhury *et al.*[8] demonstrated a significant increase in PRL levels 30 minutes after suckling in women on DMPA as opposed to those on non-hormonal contraception.[8] Thirdly, 60% of women on injectable contraceptives develop amenorrhoea within 1 year.[9] Serial endometrial biopsies in women on DMPA show evidence of endometrial atrophy in 40% of users after 1 year.[10] It is now generally believed that progestogen-induced amenorrhoea is largely due to endometrial atrophy. Nevertheless, some investigators have suggested that progestogen-induced hyperprolactinaemia may play a role in its pathophysiology.[8] Injectable progestogens are also associated with delayed return to fertility. This delay varies between individuals, but a median conception time of 9 months from the end of DMPA has been reported, and by 24 months the cumulative conception rates with DMPA are equal to those of the intra-uterine device or barrier methods.[11] Persistent anovulation appears to be the mechanism underlying delayed return to fertility, and it is thought to be due to the very slow metabolism of the drug from its microcrystalline depot in some individuals. In these women, however, ovulation can be induced by pulsatile gonadotrophin-releasing hormone (GnRH) or gonadotrophins.[12] Therefore, since PRL can inhibit pulsatile GnRH, resulting in anovulation and amenorrhoea, it is possible that progestin-induced hyperprolactinaemia may contribute to the delayed return to fertility associated with DMPA.

The mechanism of hyperprolactinaemia due to injectable progestogens may be via a direct action on the anterior pituitary, or indirectly by inhibiting the hypothalamic secretion of dopamine. Direct action may be mediated by specific receptors that have now been recovered from cytosolic and nuclear fractions of homogenised target cells.[13]

Chronic hyperstimulation of the anterior pituitary may result in the development of prolactin-secreting pituitary adenomas because exogenous oestrogens induce lactotroph hyperplasia and prolactinomas in rats,[14] and pituitary tumour growth occurs in human pregnancy[15] and after exogenous oestrogen therapy.[16] Chronic use of injectable contraceptives may, therefore, result in the development or exacerbation of prolactin-secreting pituitary adenomas.

There is paucity of published data on the incidence and severity of progestin-induced hyperprolactinaemia and its role in the pathogenesis of amenorrhoea and delayed return to fertility, as well as its effect on the risk of developing prolactin-secreting pituitary adenomas.

Therefore, the aim of this study was to demonstrate the effect of DMPA and NET EN on basal prolactin levels, with a view to investigating the role of progestogen-induced hyperprolactinaemia in the pathogenesis of galactorrhoea, amenorrhoea and possibly prolactinomas.

METHOD

This was a collaborative study between the Departments of Chemical Pathology and Obstetrics and Gynaecology at the University of Natal. The study group was recruited from black South African women attending the Commercial Centre Family Planning Clinic (CCFPC) in Durban. DMPA and NET EN are the only forms of injectable contraception administered at this clinic. During 1997, 118 937 women attended the CCFPC. Injectable contraceptives were administered to 48% of these women. Thirty-one per cent of the women using injectable contraceptives preferred NET EN, while only 17% preferred DMPA. A total of 74 women were recruited into the study group. Of these, 39 women were using DMPA, while 35 used NET EN. The dosages for DMPA and NET EN were 150 mg and 200 mg respectively. DMPA was administered every 3 months and NET EN every 2 months. Duration of use and adverse effects, including menstrual pattern changes, were recorded.

The control group was recruited from black South African women attending the Gynaecology Outpatient Department at King Edward VIII Hospital, Durban, for ailments that do not have an effect on PRL secretion. Most women had vulval warts or had come for review after treatment of pelvic inflammatory disease. A total of 62 women were recruited into the control group.

All women were counselled, and informed consent was obtained. The following exclusion criteria were applied to both groups: current pregnancy, pregnancy in the past 3 months (including ectopics, abortions and stillbirths), breast-feeding, cessation of breast-feeding in the past 3 months, medication that affects PRL secretion (e.g. phenothiazines, thioxanthenes, butyrophenones, tricyclic antidepressants, monoamine oxidase

inhibitors, anti-tuberculosis drugs, reserpine, methyldopa, verapamil, opiates, amphetamines, diazepam, cimetidine and steroids), illicit drugs that affect PRL secretion (e.g. cocaine, opiates) and medical illnesses that affect PRL secretion (e.g. thyroid disease, epilepsy, known pituitary tumours, spinal cord disease, chronic renal failure, liver disease and surgery within the past 3 months). Non-lactating postpartum women were only selected if they had had spontaneous return of menstruation. Women who had used other forms of hormonal contraception in the past 3 months were also excluded from the study group. Those who had used any form of hormonal contraception in the past 3 months were excluded from the control group.

Blood samples were obtained by venepuncture between 09h00 and 11h00. Serum PRL levels were determined by the '1-step sandwich' enzyme-linked immunosorbent assay (ELISA) on the ES-700 multiple channel chemistry analyser (Boehringer Mannheim). This is a direct immunometric assay that employs highly specific monoclonal antibodies and is capable of detecting serum PRL levels between 21 and 8 000 μIU/ml. The normal reference range for this method was 83 - 586 μIU/ml. There is no measurable cross-reactivity with human chronionic gonadotrophin, growth hormone, thyroid stimulating hormone, follicle stimulating hormone and luteinising hormone.

Student's *t*-test was used for statistical analysis.

RESULTS

Basal serum PRL levels

Basal serum PRL levels ranged from 73 μIU/ml to 786 μIU/ml in the study group, and from 42 μIU/ml to 570 μIU/ml in the control group. The mean PRL level in the study group was 266 μIU/ml (standard deviation (SD) 141) compared with 245 μIU/ml (SD 131) in the control group. Thirty-four women (46%) in the study group had PRL levels that were higher than the mean, compared with 27 (44%) in the control group. There was no significant difference between the two groups ($P = 0.39$).

Table I. Basal serum prolactin levels in study and control groups

	Study group ($N = 74$)		
	DMPA ($N = 39$)	NET EN ($N = 35$)	Control group ($N = 62$)
No.	39	35	62
Mean prolactin (μIU/ml)	226 (SD 101)	310 (SD 165)	245 (SD 131)
P-value	0.43	0.03	–

DMPA = depot medroxyprogesterone acetate; NET EN = norethisterone oenanthate; SD = standard deviation.

The mean PRL level in women using DMPA was 226 μIU/ml (SD 101) compared with 310 μIU/ml (SD 165) in those using NET EN (Table I). Sixteen (41%) and 21 (88%) women on DMPA and NET EN respectively had PRL levels that were higher than the mean. There was no significant difference between the PRL levels in the control group and those on DMPA ($P = 0.62$). There was, however, a statistically significant difference in basal serum PRL levels between the control group and women on NET EN ($P = 0.03$).

Duration of use

The mean duration of use among women on DMPA was 33 months (SD 35), compared with 32 months (SD 36) among women on NET EN (Table II). There was no significant difference in the duration of use between women on DMPA and those on NET EN ($P = 0.97$).

Table II. Clinical characteristics in the study and control groups

	Study group ($N = 74$)		
	DMPA ($N = 39$)	NET EN ($N = 35$)	Control group ($N = 62$)
Mean age (yrs)	28 (SD 6)	26 (SD 6)	30 (SD 9)
Mean parity	1.8 (SD 1)	1 (SD 1)	1.5 (SD 1)
Mean duration (mo.)	33 (SD 35)	32 (SD 36)	–

DMPA = depot medroxyprogesterone acetate; NET EN = norethisterone oenanthate; SD = standard deviation.

Age and parity

Ages ranged from 18 to 48 years in the study group, and from 14 to 59 years in the control group. The mean age in the study group was 27 years (SD 6), and that for the control group was 30 years (SD 9). Among the women on DMPA and NET EN the mean ages were 28 years (SD 6) and 26 years (SD 6) respectively (Table II). Women using DMPA also had a higher mean parity of 1.8 (SD 1) compared with 1 (SD 1) in the NET EN group (Table II). There was no significant difference in age ($P = 0.07$) and parity ($P = 0.2$) between women on DMPA and those on NET EN.

Acceptability and efficacy

Acceptability of DMPA and NET EN in the study group was reasonably satisfactory. This was also accompanied by a very high compliance rate. Only 4 of the 74 women (5.3%) in the study group had a past history of defaulting. The efficacy for both drugs in our study group was 100%. There were no pregnancies reported in the 53 women who had used injectable contraceptives for more than 12 months. However, acceptability and efficacy could not be properly assessed in this study since women who found the contraceptive method unacceptable may have either defaulted or switched to another

form of contraception. Moreover, women who fell pregnant while on injectable contraceptives are unlikely to choose the same mode of contraception after delivery.

Adverse effects

The only adverse effects reported were galactorrhoea, abnormal menstruation, amenorrhoea and vaginal discharge (Table III). Only 1 woman complained of galactorrhoea. She was 25 years old with a parity of 1 and had been using DMPA for 6 years. Galactorrhoea was her only complaint. She had no significant past medical history, no recent pregnancy, and denied use of any medications or illicit drugs. Other than the demonstration of galactorrhoea, findings on examination were essentially normal. Her serum prolactin level was 202 μIU/ml. Galactorrhoea was very disturbing to this woman and she chose to switch to another form of contraception.

Table III. Adverse effects

	DMPA ($N = 39$)	NET EN ($N = 35$)	Total ($N = 74$)
Galactorrhoea (%)	1 (3)	–	1 (1)
Abnormal uterine bleeding (%)	11 (28)	7 (20)	18 (24)
Amenorrhoea (%)	21 (54)	9 (26)	30 (41)
Vaginal discharge (%)	7 (18)	2 (6)	9 (12)

DMPA = depot medroxyprogesterone acetate; NET EN = norethisterone oenanthate.

Eighteen women (24%) complained of prolonged or irregular menstruation, while 41 women (55%) had amenorrhoea. Prolonged or irregular bleeding was restricted to women who had used injectable contraceptives for less than 12 months. The frequency of amenorrhoea depended largely on the duration of use. Of the 27 women who had used DMPA for more than 12 months, 15 (56%) had amenorrhoea, compared with 8 (32%) of the 25 who used NET EN for more than 12 months. Amenorrhoea occurred in 6 (27%) of the 22 women who had used injectable contraceptives for less than 12 months. The mean PRL level in women who had amenorrhoea was 286 μIU/ml (SD 155), compared with 252 μIU/ml (SD 131) in those who did not develop amenorrhoea. There was no significant difference between these two groups of women ($P = 0.32$). There was also no significant difference in serum PRL levels between women who did and those who did not develop amenorrhoea while on NET EN ($P = 0.10$).

Three women complained of foul-smelling yellow or greenish vaginal discharge that required antibiotic treatment. Six women, all on DMPA, complained of odourless, colourless vaginal discharge. Three preferred to switch from DMPA to NET EN because of persistent vaginal discharge. None of the women in the study group preferred to switch from NET EN to DMPA or to discontinue injectable contraception as a result of persistent vaginal discharge.

Discussion

Galactorrhoea was a relatively uncommon side-effect and manifested in only 1 woman. PRL remained within the normal range in this woman. Like other pituitary hormones, PRL exhibits marked molecular heterogeneity, accounting for its different molecular forms (little, big and big big PRL) and different levels of bioactivity. Little PRL is a non-glycosylated monomer (23 000 Dalton (D)) and has the greatest bioactivity and immunoactivity. It also has a high affinity for PRL receptors.[17] Glycosylation results in two other molecular forms (25 000 D each), with reduced immunoactivity. 'Big PRL' (50 000 D) consists of dimeric and trimeric glycosylated forms. 'Big big PRL' (100 000 D) consists of glycosylated PRL coupled with an immunoglobin. This results in poor receptor-binding affinity and low bioactivity.[18] Women with high levels of circulating PRL detected by radio-immunoassay may be asymptomatic. This is because of the structural heterogeneity of PRL, such that high levels of bioactive PRL may occur in the presence of low levels of immunoreactive PRL.[19] Consequently only 33% of hyperprolactinaemic patients have galactorrhoea. Likewise, galactorrhoea may occur in patients with normal PRL levels.[20] This was the case in our patient. Progestins usually act as oestrogen antagonists and high levels of PRL may fail to stimulate milk production in hypo-oestrogenic states. However, this is an unlikely explanation for the low incidence of galactorrhoea in our study group because women on DMPA maintain early follicular-phase oestradiol levels and normal gonadotrophin levels, and do not have menopausal symptoms.[21]

Amenorrhoea occurred in 41% of the women in the study group. More women on DMPA (54%) had amenorrhoea compared with those on NET EN (26%). Amenorrhoea was closely related to the duration of contraceptive use. Basal serum PRL levels were not associated with the development of amenorrhoea. This was also the case in women on NET EN. Consequently hyperprolactinaemia *per se* is an unlikely explanation for amonorrhoea in women on injectable contraceptives. Furthermore, basal serum PRL levels cannot be used to predict the development of amenorrhoea.

DMPA was introduced into South Africa in the mid 1960s and NET EN much later. As such older women have much more experience with DMPA than with NET EN. Additionally, the return to fertility is much less affected by NET EN, but contraceptive efficacy is greater with DMPA. Younger women who may require quick return to fertility would therefore prefer NET EN, while older patients who have completed their families would prefer DMPA. In this study, although there was a tendency for older women to prefer DMPA, this was not statistically significant ($P = 0.07$).

PRL secretion is affected by certain physiological conditions. Levels increase 2 - 3 hours after the onset of sleep and following various stimuli, including stress, afternoon meals

(especially if high in protein), venepuncture, coitus and breast and pelvic examination. Lowest levels occur between 08h00 and 12h00.[18] In addition to this diurnal variation, PRL secretion is characteristically pulsatile. In this study samples were obtained strictly between 09h00 and 11h00. Similar resting conditions prevailed during venesection, and patients who had acute or severe illnesses and those who had had surgery in the past 3 months were excluded from the study.

Although the study was not able to evaluate directly the relationship between PRL levels and delayed return to fertility, and the sample size was too small to investigate the incidence of prolactin-secreting pituitary adenomas, two important deductions can be made. Since we found no significant increase in basal PRL levels in women on DMPA, increased PRL levels are unlikely on cessation. Increased PRL secretion *per se* is therefore an unlikely mechanism for delayed return to fertility in women on DMPA. Additionally, since there was no significant increase in PRL secretion, the degree of chronic pituitary hyperstimulation is minimal. As such the effect of DMPA on the incidence of prolactin-secreting pituitary adenomas is probably insignificant. On the other hand, women on NET EN had significantly elevated basal PRL levels, although these remained within the normal range. In these women chronic hyperstimulation of the anterior pituitary may be significant, and may predispose them to prolactin-secreting pituitary adenomas. The risk, however, is probably minimal.

To the best of our knowledge, this is the first study to investigate the effect of DMPA and NET EN on PRL secretion in South African women. We found that the use of DMPA does not significantly affect basal serum PRL levels. This is in agreement with the study by Jeppsson *et al.*[21] that evaluated the effect of DMPA on the hypothalamo-pituitary-gonadal axis in nine 26 - 41-year-old amenorrhoeic women. The duration of use varied from 52.8 to 127.2 months (mean 106.8 months). The basal PRL levels remained within normal limits. The investigators concluded that long-term use of DMPA (even up to 10 years) does not induce hormonal changes different from those seen after the very first injection. However, published reports on the effect of NET EN on basal serum PRL levels seem to be scanty.

The reason why DMPA does not affect basal PRL secretion while NET EN does, is not clear. It is possible that DMPA may cause increased secretion of more bioactive PRL without affecting total basal serum PRL. Women on DMPA would therefore be symptomatic (i.e. galactorrhoea, amenorrhoea), while maintaining normal serum PRL levels. NET EN, on the other hand, may stimulate increased secretion of all forms of PRL, resulting in elevated serum PRL levels while producing a similar though less marked effect. Future studies in which the different forms of PRL are measured in women on injectable contraceptives will help to clarify this issue.

Another possible explanation for lack of association between PRL levels and amenorrhoea may be related to the pulsatile secretion of PRL. Episodic PRL secretion is frequently synchronous with that of luteinising hormone (LH). The synchrony is either simultaneous, or the PRL peak follows or is preceded by the LH peak by 10 minutes. The pathophysiology of this co-pulsatility is ill-understood. However, the time lag may reflect coupling between PRL and relevant secretagogues or inhibitors, e.g. dopamine and GnRH-associated peptide.[22] Such interaction may reflect an intrapituitary paracrine relationship between lactotroph and gonadotroph cells.[23] Furthermore, abnormal pulsatile secretion of PRL in relation to the LH peak may cause anovulation, and transient nocturnal hyperprolactinaemia may induce luteal insufficiency and galactorrhoea.[24] Asukai *et al.*[25] found that almost all infertile women with an exaggerated response to thyrotrophin-releasing hormone exhibited galactorrhoea, oligomenorrhoea, amenorrhoea, luteal insufficiency or anovulation. These investigators concluded that women with 'occult hyperprolactinaemia' have a high potential for prolactin secretion and are at risk of amenorrhoea and anovulation. It is possible that injectable contraceptives may induce abnormal pulsatile secretion of PRL and hence play a role in the pathogenesis of both galactorrhoea and amenorrhoea. Further studies are required to clarify this issue.

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