EFFECT OF THE COMBINED STEROIDAL CONTRACEPTIVES (GYNANOVLAR 21 & LYNDIOL 2.5) ON THE HUMAN ADRENOCORTICAL AND OVARIAN ACTIVITIES

By

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INTRODUCTION

The widespred use of long term cyclic treatment with progestogen oestrogen combinations for contraceptive purposes has raised many questions as yet unanswered as regards their effects on the steroid producing endocrine glands, mainly the adrenal cortex and the ovary. As regards the activity of the adrenal cortex, controversy has continued to exist concerning the concentration of plasma cortisol during oestrogen treatment as well as contraceptive therapy.

Oestrogen treatment was claimed to be associated with increased plasma cortisol level (Fukui et al, 1961, Kitay, 1964, Tait et al, 1964, Plager et al, 1964, Sandberg et al, 1966, Murray, 1967, Richard et al, 1969), with normal concentration (Doe et al, 1960, Mills et al, 1960), as well as with decreased adrenal secretion rate (Vogt, 1955, Halzbauer, 1957, McKerns, 1967, Troop & Possanza, 1962, Cushman, 1965).

Oral contraceptives were found to be associated with elevated level of plasma 17-hydroxycorticoids (Layne et al, 1962, Metcalf & Beaven, 1963, Zinnemann et al, 1967, Maureen et al, 1969) as well as with a decreased cortisol production rate (Pincus et al, 1966).

From the above mentioned effects of contraceptive pills, it was thought to carry a preliminary study as a trial to reveal the effects of 2 types of pills mainly Gynanovlar 21 (norethisterone acetate 3 mg + ethiyl oestradiol 0.05 mg) and Lyndiol 2.5 (Lynoestrenol

2.5 mg + mestranol 0.075 mg) on the human adrenal cortex. These 2 types were chosen as they are widely used nowadays, and the first had shown a moderate and the latter the highest stimulatory effect on the adrenal cortex in experimental animals when administered in low doses for short periods (Zaki et al, 1972). The present study included the determination of plasma 17-hydroxycorticoids and 24 hour urinary excretion of 17-ketosteroids, and 17-ketogenic steroids in pill users after different periods (3, 6 & 12 months).

As oestrogens affect plasma cortisol as previously mentioned and as contraceptive pills affect the ovarian activity to a certain extent so to clear the hormonal status in pill users, as well as the effect of administration of these synthetic oestrogen-progestogen combinations, on endogenous oestrogen production, 24 hour urinary oestriol determinations were carried out. In addition, vaginal smears and endometrial biopsies were done to every patient. Also 24 hour urinary pregnandiol output was estimated to determine their effects on plasma progesterone.

As the liver plays the most important role in the metabolism of steroids, it was thought also to carry out liver function tests in all cases. This was done by estimating serum glutamic oxalacetic transaminase and serum glutamic pyruvic transaminase.

MATERIAL AND METHODS

Research Population :

Fifty women from Ghamra Army Hospital were selected for this study. All were healthy with no hepatic or renal troubles, with no apparent endocrinopathies, or family history of diabetes. Their clinical data are presented in table 1.

40 cases were on contraceptive pills for variable periods between 3 and 12 months, the other 10 cases not using any hormonal pills were used as controls. The controls were successfully matched with the test groups in relation to age, parity and body weight.

Drug and Dosage :

20 women were on Gynanovlar 21 for variable periods (3, 6 & 12 months). The other 20 cases were on Lyndiol 2.5 for the same duration of time. Pills were received on the 5th day of the menstrual cycle daily for 21 days in case of Gynanovlar 21, and for 22 days in case of Lyndiol 2.5 followed by 7 days rest.

TABLE 1

Charecteristics of the control and test groups

	Control group	Test group	G 21 users	L 2.5 users
Number of Women :	10	40	20	20
Age distribution :				
Between 20—25 years	6	26	9	17
Between 26—30 years	4	14	11	3
Parity : Number of labours :				
1 — 5	6	30	16	14
6 — 10	4	10	4	6
Number of abortions :				
No abort.	5	18	10	8
1 — 3	5	22	10	12
Average body weight in kgm.	69.3	69.4	70.3	68.45

Classification of subjects

	Gynanovlar 21			Lyndiol 2.5			Controls
	3	6	12 M	3	6	12	
No. of cases	5	5	10	5	5	10	10
Per cent	10%	10%	20%	10%	10%	20%	20%

All women were asked to attend the clinic at 8—9 a. m. (to avoid diurnal variation in plasma cortisol), on day 20—22 of the cycle (after having received 15—17 pills during the month), with the total 24 hour urine collection of the day before. Non cooperative patients were admitted to hospital for 24—48 hours to collect the urine by the help of a nurse (10 cases out of the total).

30 ml. of blood were taken from the ante-cubital vein in dry clean heparinised tubes, centrifuged, and the plasma was kept in the deep freeze for determination of total plasma 17-hydroxycorticoids (17-OHCS) by a method slightly modified from that of Peterson et al (1957). Another 2 ml. of blood were withdrawn from the same vein in another tube, allowed to clot at 37 C, then centrifuged to separate

the serum for determination of serum glutamic oxalacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) which were done by the method of Reitman & Frankel (1957). The estimations were done on the same day of withdrawal. The 24 hour urine collections were used for the determination of oestriol, pregnandiol, 17-ketosteroids (17-KS), and 17-ketogenic steroids (17-KGS). Oestriol estimation was done by a method slightly modified from that of Eberlein et al (1958), and pregnandiol by the method of Klopper (1955). The 17-KS and 17-KGS were measured by the method of Norymberski et al as modified by Diczfalusy et al (1958).

From each case, endometrial biopsy was taken and stained by hæmatoxylin and eosin, and a vaginal smear stained by papanicolaou.

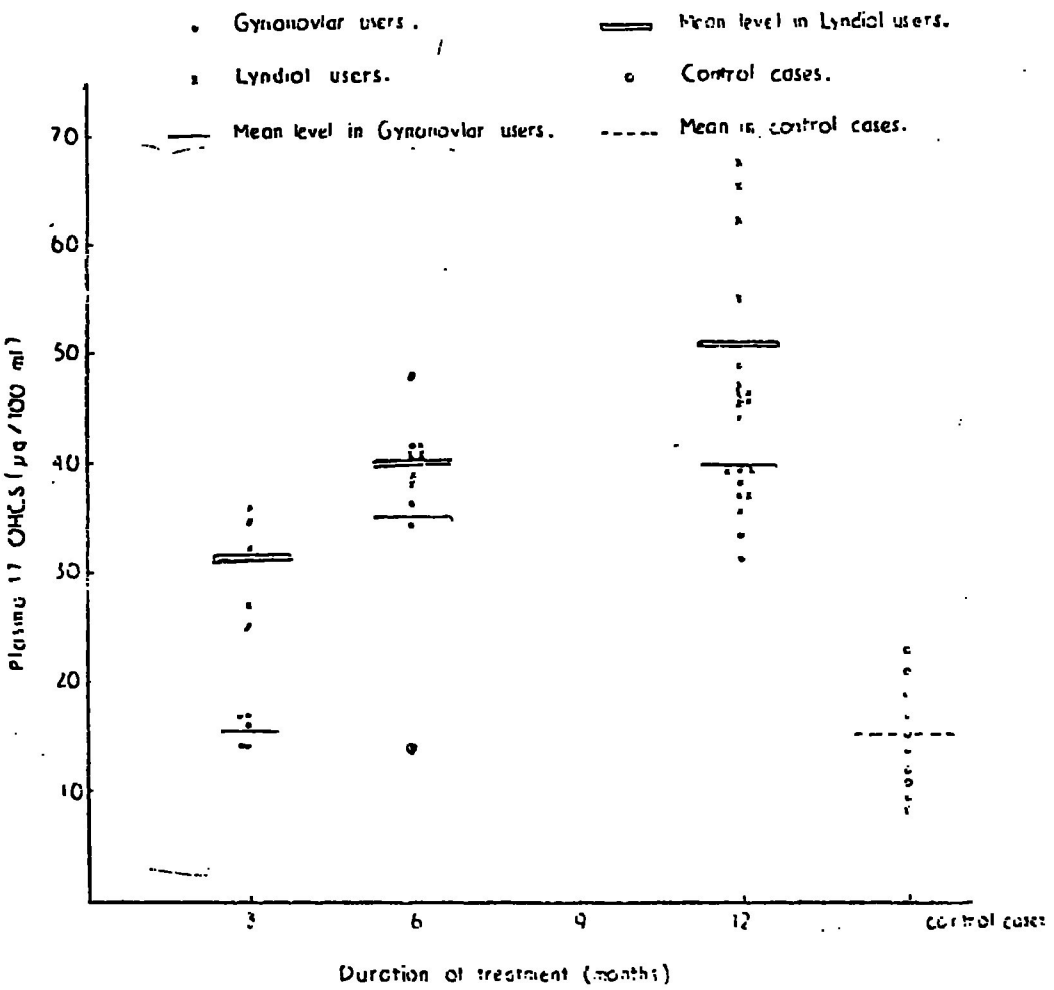

Figure (1) Total plasma 17 OHCS in subjects treated with Gynonovlar 3 and Lyndiol 2.5.

RESULTS

Plasma total 17-OHCS (Table 2) :

The mean level of plasma 17-OHCS in the groups treated with Gynanovlar 21 for 3, 6, and 12 months were respectively 15.46 ± 1.26, 34.78 ± 12.79 and 39.76 ± 5.73 ug/100 ml. In subjects treated with Lyndiol 2.5 the corresponding results were respectively 31.28 ± 5.03, 40.0 ± 1.5 and 50.61 ± 11.15 ug/100 ml plasma. These values were higher than the mean control value (15.05 ± 4.16 ug/100 ml). To show the effect of duration of treatment, the results of 3 month users were compared with those of 6 and 12 month users, and it was found

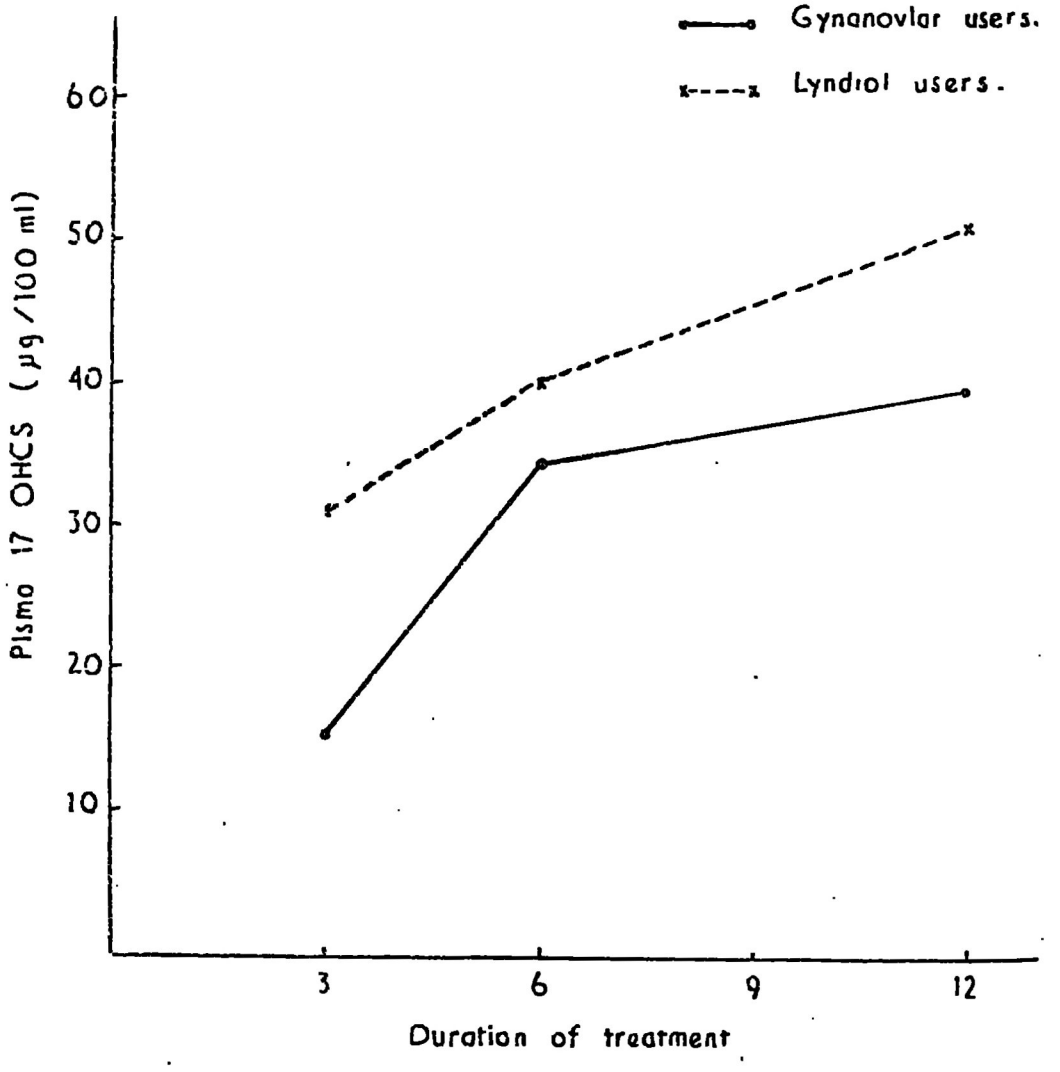

Figure. (2) Mean total plasma 17 OHCS in Gynanovlar 21 and Lyndiol 2.5 users.

TABLE 2

Statistical comparison using Fischer's method for comparing the mean results of the test groups and the control group, and to show the effect of the duration of treatment with Gynanovlar 21 and Lyndiol 2.5 on total plasma 17 hydroxy corticoids.

17 OHCS.	Mean ug/100 ml.	S. D.	Comparison	t	p	Significance
In control cases :	15.05	± 4.164				
In Gynanovlar 21 users :			3 M. & control	0.215 >	0.50	N.S
— For 3 months	15.46	± 1.26	3 M. & 6 M.	3.358 <	0.02	S
— For 6 months	34.78	± 12.79	6 M. & control	4.55 <	0.01	S
— For 12 months	39.76	± 5.73	3 M. & 12 M.	9.201 <	0.01	S
In Lyndiol 2.5 users :						
— For 3 months	31.28	± 5.03	3 M. & control	6.65 <	0.01	S
— For 6 months	40.00	± 1.50	3 M. & 6 M.	3.725 <	0.01	S
— For 12 months	50.61	± 11.14	3 M. & 12 M.	3.63 <	0.01	S

S = Significant difference

N. S. = No significant difference.

that plasma 17-OHCS increased gradually with the duration of treatment, and the difference was statistically significant in both cases (P = 0.02 & 0.01 respectively). However, comparing the results of 3 months with that of the controls the difference was insignificant (P = 0.5).

In case of Lyndiol 2.5, the difference was statistically significant when comparing the results of 3 month users with those of the controls, as well as when comparing the 6 month and 12 month users with the 3 month users (P = 0.01 in all cases). In general the values obtained in Lyndiol treated subjects were higher if compared with those of the corresponding groups on Gynanovlar 21 (Figs 1 & 2).

Urinary 17-KS (Table 3) :

In Gynanovlar and Lyndiol users urinary 17-KS were less than the mean control value. However, the values obtained in Gynanovlar users were higher than in Lyndiol users (Figs. 3 & 4). It was also

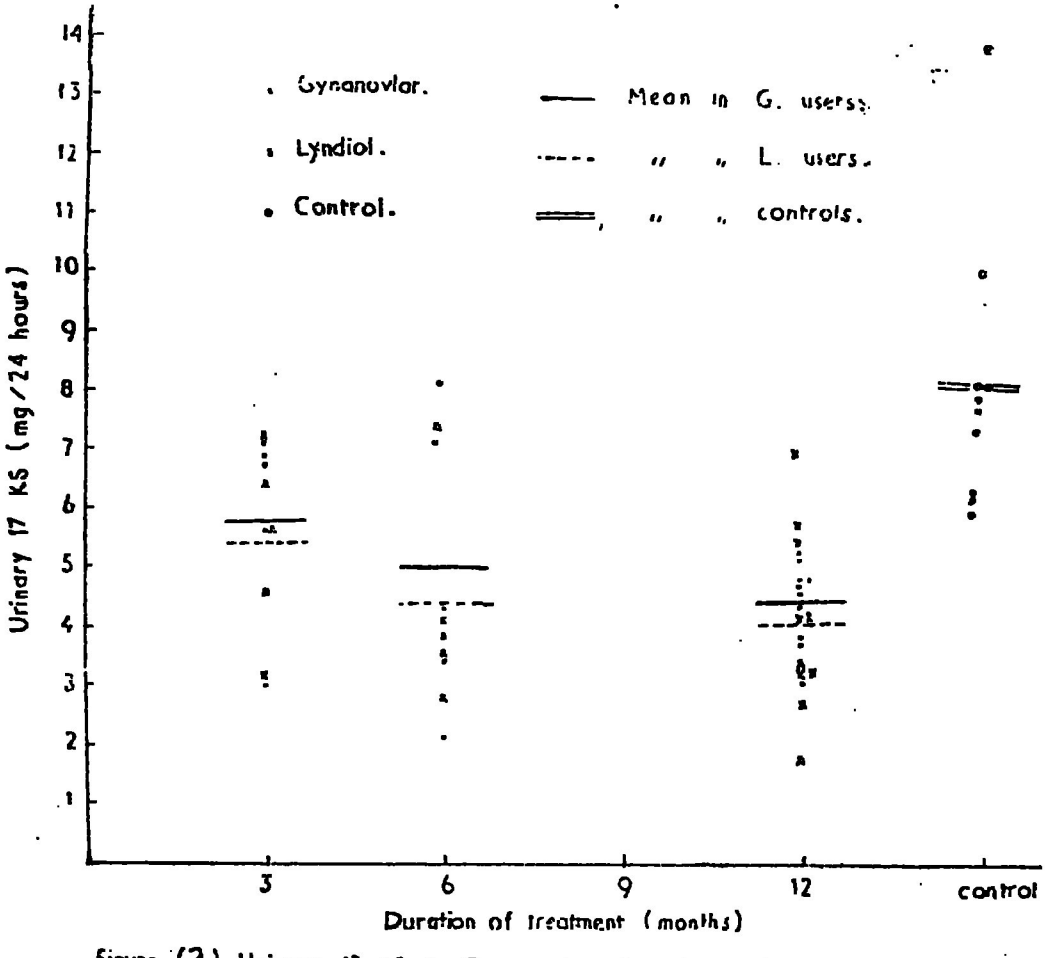

Figure (3) Urinary 17 KS in Gynanovlar 21 and Lyndiol 2.5 users.

TABLE 3

Statistical comparison using Fischer's method for comparing the mean results of the test groups and the control group, and to show the effect of the duration of treatment with Gynanovl 21 and Lyndiol 2.5 on urinary 17 ketosteriods.

17 KS	Mean mg/24 hours	S. D.	Comparison	t p	Signifi-cance
In control cases	8.49	2.543			
In Gynanovlar 21 users :					
— For 3 months	5.84	1.69	3 M. & control	2.09 > 0.05	S
— For 6 months	5.04	2.54	3 & 6 months	0.58 > 0.50	N.S
— For 12 months	4.403	0.87	3 & 12 months	2.201 < 0.05	S
In Lyndiol users :					
— For 3 months	5.40	1.56	3 M. & control	2.48 < 0.05	S
— For 6 months	4.36	1.81	3 & 6 months	1.031 < 0.50	N.S
— For 12 months	4.05	1.56	3 & 12 months	1.586 > 0.50	N.S

S = Significant difference.

N. S. = No significant difference.

noticed that the mean values obtained both in Gynanovlar users and Lyndiol users decreased gradually with the duration of treatment, being higher in 3 month than in 12 month users (Fig. 4). The highest mean value was in Gynanovlar users for 3 months, and the lowest values obtained in Lyndiol users for 12 months.

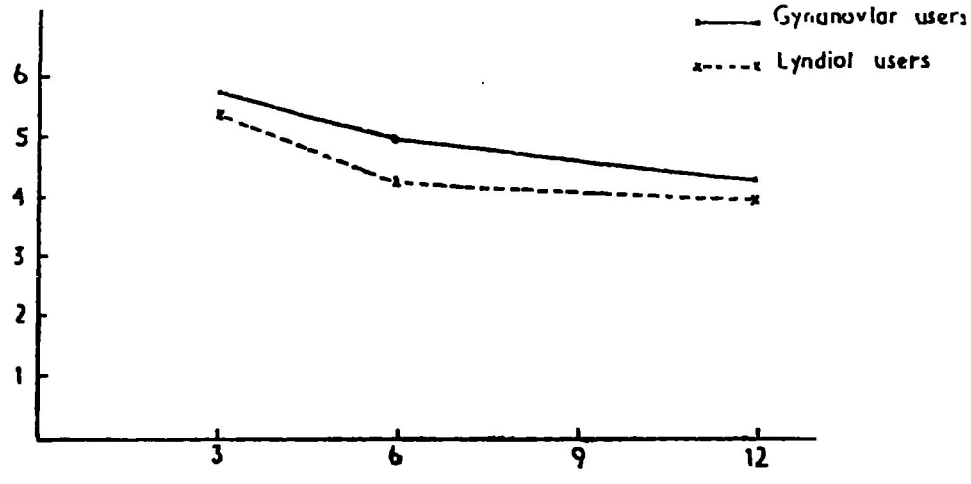

Figure (4) Mean urinary 17 KS in Gynanovlar and Lyndiol users.

On comparing the mean control value with the mean of 3 month Gynanovlar users, the difference was statistically significant (0.05), while on comparing the results of 6 and 12 month users with those of 3 months, the first was not significant (P = 0.5), and the latter was significant (P = 0.05). In case of Lyndiol, the difference between 3 month users and the mean control value was significant (P = 0.05), while the difference between 3 month users and those of 6 and 12 month users was statistically insignificant (P = 0.5 & 0.5).

This statistical comparison shows that urinary 17-KS were less in all the groups using Gynanovlar and Lyndiol and that their level is more or less not related to the duration of therapy.

Urinary 17-KGS (Table 4) :

In contrast to the 17-KS, the rsults of 17-KGS showed much variation between Gynanovlar and Lyndiol users, as well as in the different groups treated with Gynanovlar for different periods of time. The mean levels of 17-KGS with Gynanovlar for 3, 6, and 12 months were 20 $\pm$ 2.08, 10.44 $\pm$ 3.57 and 7.95 $\pm$ 1.24 mg/24 hours

TABLE 4

Statistical comparison using Fischer's method for comparing the mean results of the test groups and the control group, and to show the effect of the duration of treatment with Gynanovlar 21 and Lyndiol 2.5 on urinary 17 ketogenic steriods.

17 KGS	mg/24 hours Mean	S. D.	Comparison	t	p	Significance
Control cases	9.74	2.787				
Gynanovlar 21 users :						
— For 3 months	20.00	2.080	3 M. & control	7.22	< 0.01	S
			3 & 6 months	5.17	< 0.01	S
— For 6 months	10.44	3.570	6 M. & control	0.419	> 0.50	N.S
— For 12 months	7.95	1.240	12 M. & control	1.860	< 0.10	N.S
			3 & 12 months	14.045	< 0.01	S
Lyndiol 2.5 users :						
— For 3 months	7.76	1.530	3 M. & control	1.460	< 0.10	N.S
			3 & 6 months	5.380	< 0.01	S
— For 6 months	2.54	1.530	6 M. & control	5.310	< 0.01	S
— For 12 months	4.55	2.930	3 & 12 months	2.228	< 0.05	S

N. S. = Not significant.

S = Statistically significant difference.

respectevely. In Lyndiol users for 3, 6 and 12 months results obtained were 7.74 ± 1.53, 2.54 ± 1.53 and 4.54 ± 2.93 mg/24 hours respectively. In the control group, the mean value was 9.74 2.79 mg/24 hrs. This shows that in Gynanovlar treated groups for 3 and 6 months, the values were above the normal mean value. In 12 month users, it was slightly less than the average normal value. But in Lyndiol users, all values obtained were less than that of the control group, the least values obtained were in the 6 month users (2.54 ± 1.53 mg/24 hrs.).

Comparing the mean values obtained in the Gynanovlar and Lyndiol users for 3, 6 and 12 months, it was clear that in the 3 and 6 month users, the Gynanovlar group was more than double the results of Lyndiol treated group. Similarly in 12 month users, the results of the Gynanovlar groups were much higher (Figs. 5 & 6).

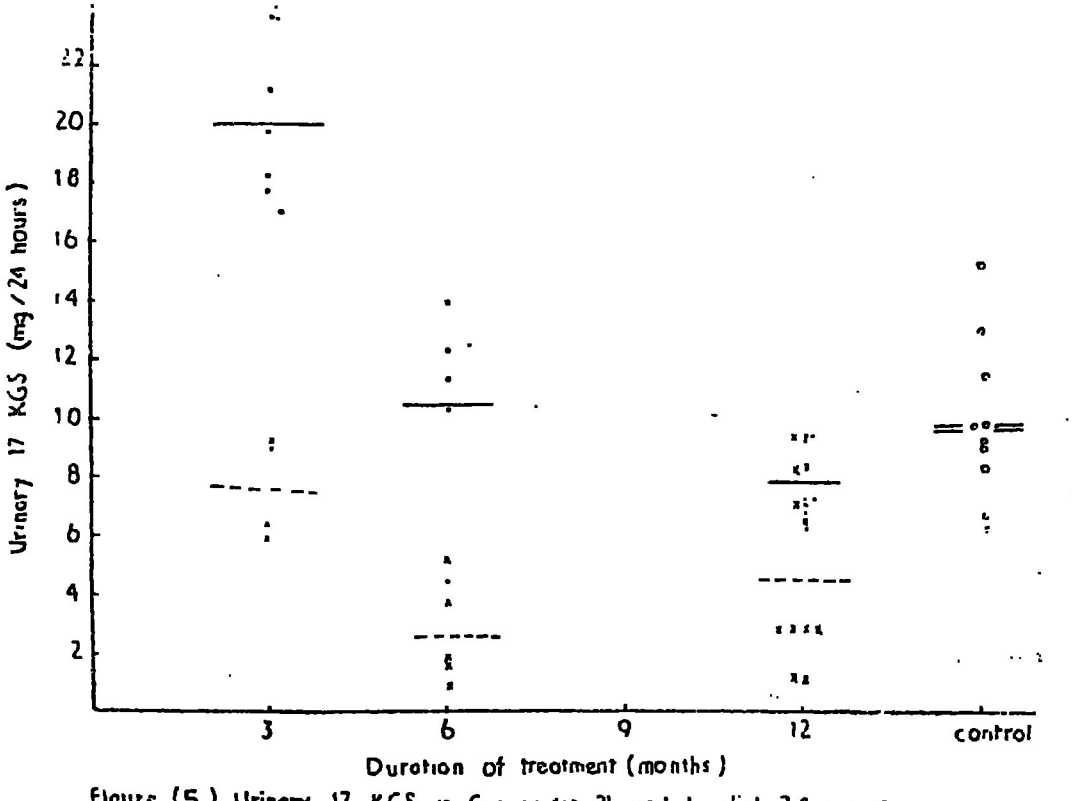

Figure (5) Urinary 17 KGS in Gynanovlar 21 and Lyndiol 25 users.

As regards the effect of duration of treatment, it was found that in Gynanovlar users, the results were lesser as the duration increased. Comparing the results of 3 moth users with those after 6 and 12 months, it was found to be statistically significant (P ± 0.01 in both).

Also when comparing the results of 3 month users with these of the controls the difference was statistically significant (P = 0.01) but when comparing 6 and 12 month users with the controls the difference was insignificant (P = 0.5 and 0.1 respectively).

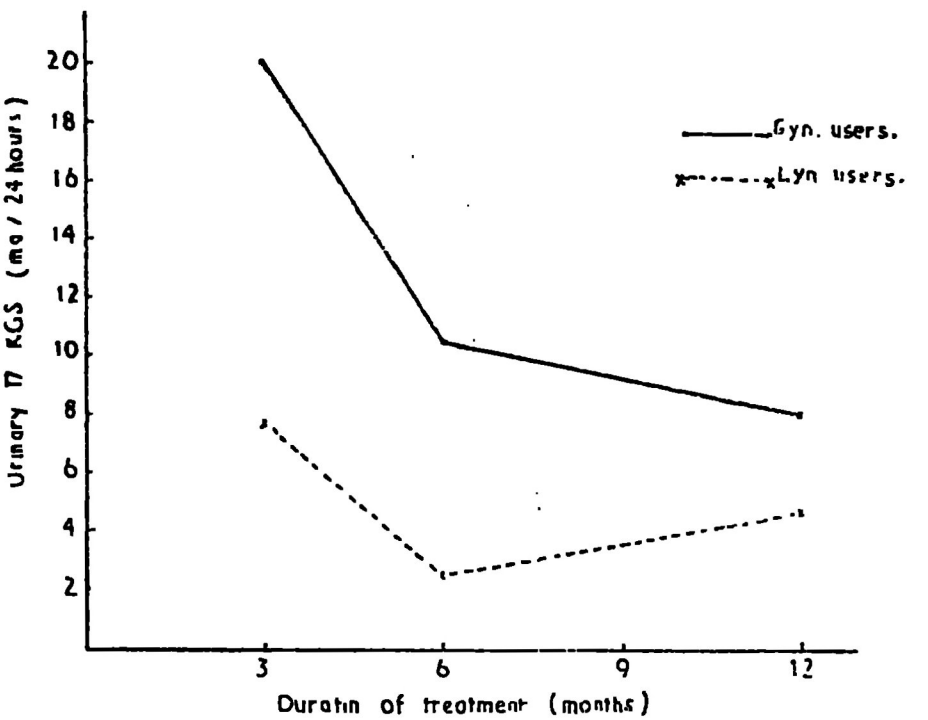

Figure (6) Mean urinary 17 KGS in pill users

In Lyndiol users, the lowest results were in 6 month users and by the same comparison between 3 month users and the results of 6 and 12 month users, it was statistically significant (P = 0.01 in both). But when comparing the results of 3 month users with the controls, the difference was insignificant (P = 0.1) and became significant when comparing 6 month users with the control (P = 0.01).

Urinary oestriol (Table 5) :

The mean urinary oestriol levels in 24 hour urine collections from Gynenovlar users for 3, 6 and 12 months were 10.56 ± 2.87, 12.7 ± 2.53, and 5.57 ± 1.47 ug/24 hrs. respectively. In Lyndiol users the corresponding values were 11.28 ± 0.65, 8.98 ± 2.07 and 8.12 ± 1.86 ug/24 hrs. In the control group the mean value was 13.69 ± 1.99 ug/24 hrs. This shows that urinary oestriol in the cases treated with oral contraceptive steroids showed comparatively low levels if compared with untreated cases in the same period of the menstrual cycle (day 20—22 of the cycle). Comparing the values

TABLE 5

Statistical comparison using Fischer's method for comparing the mean results of the test groups and the control group, and to show the effect of duration of treatment with Gynanovlar 21 and Lyndiol 2.5 on 24 hours urinary oestriol.

(ug/24 hours)

Oestriol	Mean ug/24 h.	S. D.	Comparison	t	p	Signi-ficance
Control	13.69	1.987				
Gynanovlar 21 :						
— 3 months	10.56	2.87	3 M.+ control	2.495	< 0.05	S
			3 M.+ 6 M.	1.247	< 0.50	N.S
— 6 months	12.70	2.53	6 M.+ control	2.17	< 0.05	S
			6 M.+ 12 M.	6.96	< 0.01	S
— 12 months	5.57	1.48	3 M.+ 12 M.	4.560	< 0.01	S
			12 M.+ control	10.25	< 0.01	S
Lyndiol 2.5 :						
— 3 months	11.28	0.66	3 M.+ control	2.59	< 0.05	S
			3 M.+ 6 M.	2.345	< 0.02	S
— 6 months	8.98	2.07	6 M.+ control	4.26	< 0.01	S
— 12 months	8.12	1.86	3 M.+ 12 M.	3.622	< 0.01	S

obtained in Gynanovlar and Lyndiol users for 3, 6 and 12 months (Figs 7 & 8) it is clear that in 3 month users the mean level was slightly less in Gynanovlar users than in Lyndiol users.

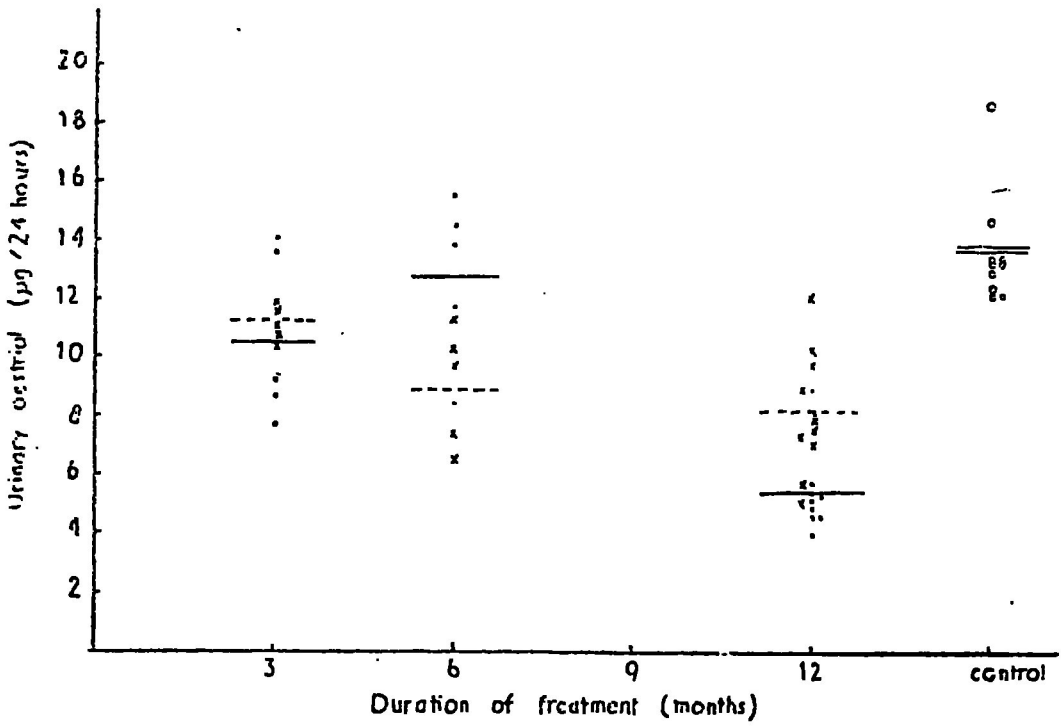

Figure (7) Urinary estriol level in patients treated with Gynanovlar 21 and Lyndiol 2.5 .

In 6 month users, the mean value was higher in Gynanovlar than in Lyndiol users. In 12 month users Gynanovlar group again recorded lower levels as in 3 month users.

From the data it is also clear that the mean level is not related to the duration of treatment in Gynanovlar users. In Lyndiol users the mean levels recorded decreased gradually with the duration of treatment, (fig. 8).

Urinary pregnandiol (Table 6) :

All the results obtained in this work were less than 1 mg/24 hrs. compared to the control group (mean 2.27 $\pm$ 0.07 mg/24 hrs.).

S. G. O. T. and S. G. P. T. (Table 6) :

Taking the normal values for SGOT 5—40 units and SGPT 1—30 units, this shows that all values obtained in this investigation were within the normal limits.

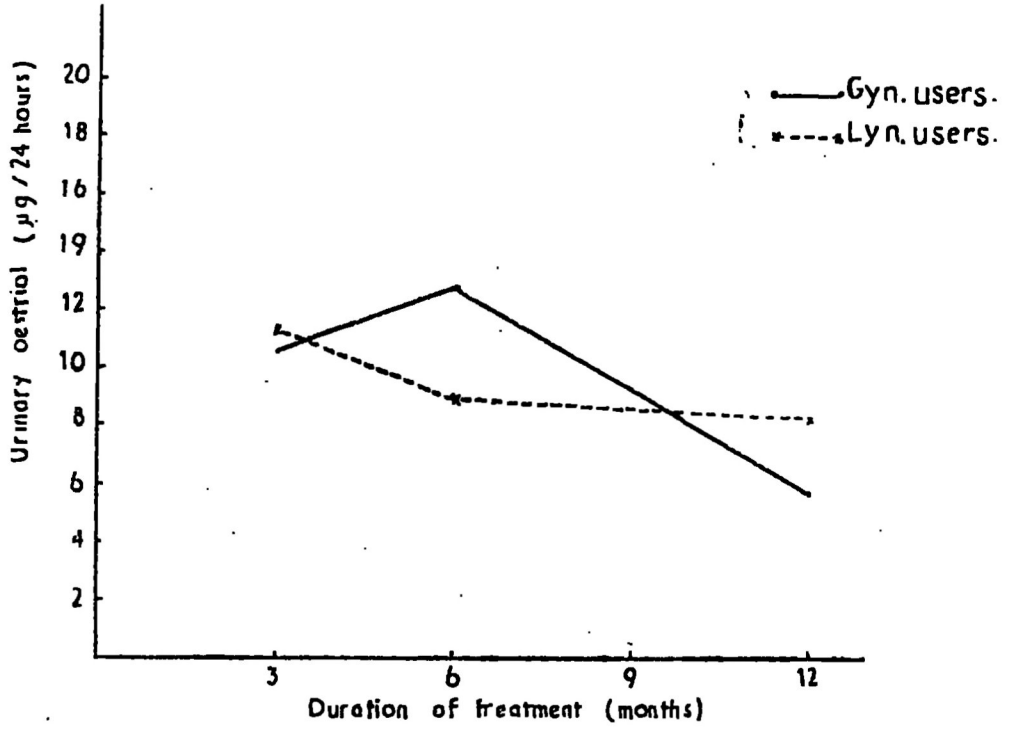

Figure (8) Mean urinary estriol level in pill users.

Vaginal Smears : The smears were similar in all the groups except in Lyndiol users for 12 months. There was a great shift to the middle with a mean intermediate cell count of 75—90 per cent in all groups. There were no parabasal cells, except in 4 cases that showed severe infection. In most of the cases the intermediate cells showed a minor degree of clumping and curling. The background was clear. In Lyndiol users for 12 months, there was a slight shift to the right, i. e. an increase in the number of superficial cells.

Endometrial biopsies : Biopsies taken on day 20—22 of the cycle showed changes more or less similar in the 6 groups investigated. In all cases except 2, it was found that :

The number of glands : few to moderate.

Epithelium : varied from low to tall columnar.

The gland cavity : tubular with no evidence of any secretion neither in the cells nor in the gland cavity.

The stroma : showed slight to moderate differenciation.

In 4 of these cases the glands showed mild cyctic changes inspite

TABLE 6

Mean 17 OHCS, 17 KS, 17 KGS pregnandiol, estriol and liver functions in pill users and control cases.

		Gynanovlar 21 users			Lyndiol 2.5 users			Control
		3 M	6 M	12 M	3 M	6 M	12 M	
Plasma	Mean	15.460	34.780	39.760	31.280	40.000	50.610	15.050
17 OHCS.	S. D.	1.261	12.790	5.730	5.030	1.502	11.145	4.164
17 KS.	S. D.	1.686	2.520	0.875	1.562	1.810	1.560	2.543
Urinary	Mean	5.840	5.040	4.403	5.400	4.360	4.050	8.490
Urinary	Mean	20.000	10.440	7.946	7.740	2.545	4.545	9.740
17 KGS.	S. D.	2.083	3.575	1.205	1.534	1.532	2.930	2.787
Urinary	Mean	10.560	12.700	5.570	11.280	8.980	8.124	13.690
estriol	S. D.	2.870	2.530	1.477	0.659	2.070	1.860	1.987
Urinary	Mean	0.560	0.796	0.784	0.600	0.824	0.797	2.270
pregnandiol	S. D.	0.125	0.177	0.146	0.022	0.215	0.101	0.069
Liver	SGOT.	12.500	11.700	21.600	9.500	18.000	15.900	17.45
function	SGPT.	20.000	16.200	19.400	22.500	11.000	19.800	16.000

of the persistance of the atrophic changes described above. These atrophic changes did not differ in quality or quantity in the different groups.

In the 2 cases excluded, the endometrium showed typical secretory manifestations in all its elements, i. e. glands were many, lined with tall epithelium, the cavity was corckscrew, and full of secretions, the stroma showed moderate cellularity and increased vascularity with areas of hæmorrhage. These findings were seen in case No. 20 using Gynanovlar for 6 months and case No. 22 using Lyndiol for 12 months.

DISCUSSION

From the present investigation, it is clear that total plasma 17-OHCS showed an increase following the administration of Lyndiol 2.5 or Gynanovlar for several periods varying from 3 up to 12 months. The mean values in Gynanovlar users were about half those obtained with Lyndiol 2.5 after 3 months, and the values of 17-OHCS after 6 and 12 months of Gynanovlar therapy were in general less than those obtained with Lyndiol for the same duration of treatment. The highest values were found in Lyndiol 2.5 users for 12 months which were much higher than the mean value of the control cases. Whether this increase in the total plasma 17-OHCS with Lyndiol 2.5 or Gynanovlar 21 was accompanied by a similar increase in the protein unbound fraction or not, this needs further investigations as the unbound biologically active fraction was not measured in the present work. However, several investigators found increased non-protein bound cortisol after oestrogen administration (Plager et al, 1964, Murphy et al, 1967, Hannan et al, 1969,). However, Doe et al, (1960), and Keane et al (1969), claimed that oestrogen administration was associated with normal concentration of non-protein bound cortisol. This was explained by the increase in transcortin binding capacity or concentration under such conditions thus causing increased total plasma cortisol (Sandberg et al, 1959, Tait et al, 1964, Layne et al, 1962).

That oral contraceptives cause a rise in plasma corticosteroids was mentioned by several investigators (Halkerston et al, 1956, Peterson et al, 1960, Besch et al, 1965, Botterman et al, 1967, Laurell et al, 1968).

Layne and Pincus (1964), found that Enovid has a marked oestrogenic effect on cortisol metabolism. Dodek et al (1965), concluded that Enovid caused raised plasma cortisol level without affecting its rate of production. But it decreased its metabolic clearance rate, possibly by increasing its binding by transcortin. The same results were found by other investigators (Wallace et al, 1957, Paulshock et al, 1966) who demonstrated decreased turn-over rate of cortisol. Martti et al, (1967), concluded that oral contraceptives increased both conjugated and free plasmacortisol levels.

Moreover, Maureen et al (1969), and David et al (1969), found increased concentration of total and free plasma cortisol after Enovid therapy.

So the rise in plasma 17-OHCS recorded in the cases examined in the present investigation may be most probably due to the oestrogen component of the pills and its effect on transcortin. So one can say that Lyndiol 2.5 being more oestrogenic than Gynanovlar 21, it caused higher elevation of total plasma 17-OHCS. This can be explained by the fact that the oestrogen component of Lyndiol 2.5 is mestranol 0.075 mg, while that of Gynanovlar 21 is ethinyl oestradiol 0.05 mg, as both have the same oestrogenic potency in equal doses, so Lyndiol containing bigger dose than Gynanovlar is expected to exert a more oestrogenic effect. In addition, the progestogen componentof Lyndiol (Lynestrenol) is well known to be readily transformed in the body to oestrogenic substances more than that found in Gynanovlar 21 (Norethisterone acetate), hence increases the oestrogenic potency of Lyndiol 2.5. Also Lynestrenol is capable of inhibiting ovulation without oestrogen addition in contrast to Norethisterone acetate which has no contraceptive effect without an added oestrogen.

However, inspite of the elevated plasma cortisol in contraceptive pill users, as well as after oestrogen administration, no symptoms of hypercorticism have been noticed.

David et al (1969), found that for Cushing syndrome to occur, high plasma cortisol level should be maintained for most or all of the day. This explains the absence of Cushing's syndrome during oestrogen administration and during contraceptive pill therapy as the cercadian variation which is known to be present under such conditions, brings it to the normal range at 9 p. m. (Richard et al, 1969). In addition the increased transcortin level keeps the physiologically active free cortisol low enough as not to produce Cushing's syndrome.

Another finding in the present investigation is that the rise in plasma 17-OHCS was gradual with the duration of treatment with either Gynanovlar or Lyndiol. This *may* be due, among several other possible factors, to accumulation of the drug or its effect especially that the metabolism of such synthetic steroidal preparations used in the combined contraceptive pills differs markedly from that of natural oestrogens and progesterone.

Williams et al (1967), found that following the ingestion of a single dose of quinestrenol, metabolites can be found in the urine during more than 4 months.

Also the progestogens of the pills have not the same effects as progesterone. The latter was shown to enhance oestrogen metabolism in the liver (Varangot & Sedar, 1957) as well as the activity of hepatic enzymes. Moreover, they constitute a load on the liver for their metabolism, a condition which participate in changing the hormonal pattern in pill users.

However, from the results obtained in the present investigation one cannot conclude delayed cortisol metabolism mentioned by other workers (Stiefel et al, 1959, Peterson et al, 1960, Mills et al, 1960, Marks et al, 1961, Yates & Urquhart, 1962, Nelson et al, 1963), with either Gynanovlar or Lyndiol. But it is still a sugesstion and the results of urinary 17-KS and 17-KGS may be of value in this respect.

Data obtained showed clerly that the increase in plasma 17-OHCS was accompanied by a decrease in urinary 17-KGS, (except with Gynanovlar 21 for 3 months), and as the latters reflect the metabolites of 17-OHCS, the decrease in 24 hour urinary excretion of 17-KGS indicates clearly either delayed metabolism in the liver due to direct effect of these exogenous steroids on the liver enzymes respomsible for corticosteroid degradation, or an increase in the concentration or binding capacity of transcortin thus rendering cortisol not available for metabolism. A third possibility is that the high levels of total plasma 17-OHCS in pill users may be an indication of a new pituitary-adrenal balance accompanied by a decrease in the secretory rate of corticoids from the adrenal cortex hence low levels of urinary 17-KGS are expected. However, Starup et al (1967) and Ostergaard et al (1966), stated that these changes are due to changes in corticosteroid

metabolism and not to affection of the pituitary-adrenal feed pack mechanism as proved by metopirone administration. Also several authors found that the response of plasma cortisol and corticosteroid excretion to ACTH is unaltered under contraceptive therapy (Apostolakis & Napp, 1960, Leach et al, 1965).

Comparing the results of 17-KGS obtained with Gynanovlar and Lyndiol, it is worth mentioning that with Lyndiol 2.5 for 3, 6 and 12 months all values were less than that of the control group. In contrast, in Gynanovlar 21 users a significant increase in 17-KGS was detected after 3 months ($P = 0.01$) and the levels were nearly normal in the 6 month group. This difference may be due to the oestrogen component of the pills. The other factor which may participate in increasing the 17-KGS with Gynanovlar is the transcortin level and its role in binding cortisol. After 3 months of Gynanovlar treatment, the level of transcortin may not be as high as with Lyndiol and hence the plasma 17-OHCS are available for metabolism. This point, however, needs further investigations.

It is worth mentioning here that with Gynanovlar treatment for 3 months the slight increase in plasma 17-OHCS was accompanied by an increase in 17-KGS, and the effects found to occur with Lyndiol 2.5 as regards the 17-OHCS and 17-KGS were not attained with Gynanovlar except after 12 months of treatment. This was also clear when comparing the rise in plasma 17-OHCS with both preparations. The rise with Gynanovlar 21 after 12 months was similar to the rise with Lyndiol 2.5 after 3 months.

As regards urinary 17-KS, it showed a decrease with either Gynanovlar or Lyndiol. However, all the values in Lyndiol users were generally less than those of Gynanovlar users for the same duration of treatment.

Several investigators reported decreased excretion of urinary metabolites of corticosteroids with pill intake, (Starup et al, 1966, Ostergaard et al, 1966, Binder et al, 1967, and Robert et al, 1967, Mestman et al, 1967).

As the main sources of urinary 17-KS are the adrenal androgens, 17-OHCS metabolites, and a small amount from ovarian androgens, one can say that the suppression of ovarian activity with pill intake accompanied by the suggested delayed metabolism of corticostevoids and consequently slight suppression of the adrenul cortex under

should mention that individual variation may play an important role and it seems better to follow up each case periodically to obtain better data.

As regards liver functions under Gynanovlar or Lyndiol therapy, the normal values of SGOT and SGPT obtained in this work exclude any bad effect on the liver by these 2 types of pills.

As regards oestriol excretion in Gynanovlar and Lyndiol users, we relied on oestriol alone as ethinyl oestradiol which is found in most oral contraceptives or resulted from demthylation of mestranol enter into the oestradiol fraction and to a lesser degree into the oestrone fraction and measured as total oestrogens, (Breuer et al, 1960, Kaiser et al, 1966, & Lauritzen, 1967).

It is apparent from the results obtained that both Gynanovlar 21 and Lyndiol 2.5 caused a certain degree of ovarian suppression as evidenced by the lower levels of urinary oestriol. In addition 24 hour urinary estimations of pregnandiol showed low values in all groups indicating absence of ovulation. These findings were confirmed by vaginal smears and endometrial biopsies examinations.

The smears examined showed a great shift to the middle denoting low oestrogen effect. However, in Lyndiol users for 12 months the vaginal smears showed a slight shift to the right, i. e. more oestrogenic effect.

Endometrial biopsies taken on day 20—22 of the cycle showed in all cases except 2, changes indicating that ovulation was inhibited and the endometrium suffered some atrophic manifestations which did not differ in quality or quantity with the type of pill used. The escape of ovulation that was found in 1 case using Gynanovlar for 6 months and another case using Lyndiol for 12 months is not peculiar to the findings of other investigators who described occasional escape of pituitary inhibition under oral contraceptive therapy, (Kist er, 1959, Greenblott & Rose, 1962, Rice-Ray, 1966).

SUMMARY

A preliminary study was done to reveal the effect of Gynanovlar 21 and Lyndiol 2.5 on the human adrenocortical activity.

50 healthy women were selected for this study, 10 were used as controls, 20 were using Gynanovlar 21 for 3, 6, and 12 months, the other 20 were on Lyndiol 2.5 for the same duration of time.

The total plasma 17-OHCS as well as 24 hour urinary excretion of 17-KS, 17-KGS, oestriol and pregnandiol estimations were done, in addition to vaginal smears and endometrial biopsies. Also SGOT and SGPT estimations were carried out to every subject.

All these investigations were done on day 20—22 of the cycle and the blood samples for 17-OHCS were taken at 8—9 a. m. to avoid diurnal variation.

Data obtained show that total 17-OHCS increased after Gynanovlar and Lyndiol therapy. The mean values recorded in Gynanovlar users were generally less than in Lyndiol users. After 3 months of using the pills, the mean result of Gynanovlar treated group was about half that of Lyndiol treated group. In Gynanovlar users for 3 months the increase was insignificant when compared with the mean control value, but significant increase occurred in 6 month users, and increased still more in 12 month users. In Lyndiol users, the increase was significant even in the 3 month users, and increased still more in 6 and 12 month users.

The increased 17-OHCS was accompanied by a decrease in urinary 17-KGS in all cases except with Gynanovlar users for 3 and 6 months. In Lyndiol users, 17-KGS decreased insignificantly after 3 months than in the control group, but the decrease was significant after 6 months, and their amount decreased more after 12 months. In Gynanovlar users there was a significant increase in 17-KGS in 3 month users, than in the control group, but there was insignificant difference between the controls and 6 and 12 month users.

Urinary 17-KS were decreased in all groups after Gynanovlar and Lyndiol therapy. The values recorded were generally less in Lyndiol users than Gynanovlar.

Urinary oestriol levels were less in all groups than in the control subjects. In Gynanovlar users, the degree of diminutuon was not related to the duration of treatment. In Lyndiol users the decrease became more evident with the increase in the duration of therapy.

Urinary pregnandiol level was found to be low in all groups except 2 cases, indicating low progesterone levels and absence of ovulation.

SGOT and SGPT were all within normal limits.

Vaginal smears showed a great shift to the middle indicating low oestrogen effect. The intermediate cells showed a minor degree of clumping and curling indicating that the progesterone effect is not fully established. However, in Lyndiol 2.5 users for 12 months the vaginal smears showed a slight shift to the right i. e. increase in the number of superficial cells.

Endometrial biopsies showed changes indicating ovulation inhibition and the endometrium suffered some atrophic manifestations which did not differ in quality or quantity with the type of pill used or duration of therapy.

The significance of these findings was discussed in view of the current literature.

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