Antinuclear Antibodies, Rheumatoid Factor and C-Reactive Protein in Serum of Normal Women Using Oral Contraceptives

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Some women who report to a hospital or arthritis clinic note exacerbation of rheumatic complaints and develop serologic abnormalities while taking oral contraceptives. The current study concerns the detection of antinuclear antibodies, rheumatoid factor and C-reactive protein in normal women using these drugs. Prospective study of 82 women before and during oral contraceptive use permitted the detection of 4 who developed antinuclear antibodies, 9 who developed rheumatoid factor and 30 who developed C-reactive protein after less than 1 year of drug use. The prevalence of positive tests was greater in a group of 210 women who were using OC than that found in a group of 174 who had never used these drugs. None of these women developed rheumatic symptoms while using oral contraceptives.

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Recently several authors have reported an association between the use of oral contraceptives and the exacerbation of rheumatic complaints or the induction of autoantibodies. The prevalence of these abnormalities has varied, dependent at least in part, upon the nature of the population of women studied. Three studies (1, 2, 3) dealing with patients seen in a clinic or hospital have described the occurrence of rheumatic symptoms, antinuclear antibodies, or LE cells during treatment with oral contraceptives. When the population group was apparently normal women studied at birth control clinics, few abnormalities were found. Dubois and associates (4) tested the serum of 30 normal women for the presence of antinuclear antibodies (ANA), rheumatoid factor and LE cell phenomena, and concluded there was no evidence that oral contraceptives (OC) stimulated or induced autoantibodies. In another study (5), the prevalence of ANA in a group of 176 women was not increased;

however, the occurrence of positive tests for rheumatoid factor was increased during the use of oral contraceptives.

The current study was undertaken in order to determine if the frequency and type of serologic abnormality detected in normal women seen at a birth control clinic during OC use differed from that observed in patients reporting to a hospital or arthritis clinic. To properly interpret test results obtained during use of oral contraceptive agents, knowledge of the pretreatment status of the women was of critical importance. In addition, duration of drug use and chemical composition of these agents has been reported to influence the clinical results. Each of these factors has been evaluated in this study of healthy young women who initiated therapy with one of the currently available forms of OC.

MATERIALS AND METHODS

Serum from 503 women between the ages of 16 and 47 was collected and assayed for the presence of antinuclear antibodies (ANA), rheumatoid factor (RF), LE cells, and C-reactive protein (CRP). In Table 1 are listed certain of the characteristics of the three groups of women included in this study. Women in Groups 1 and 2 reported voluntarily between Oct 1968 and Jan 1970 to birth control clinics of the Washtenaw County League for Planned Parenthood and the University of Michigan. The first blood specimen was collected at the time of initial physical examination. Based upon history and physical findings, each woman was considered normal at the time of entrance into this study. Ten percent of the clinic patients refused to donate an initial blood specimen. Those beginning oral contraceptives for the first time were asked to return for follow-up 3 months after starting these drugs.* The women in Group 2 were taking OC at the time of the initial examination. The Group 3 sera used in the present study represent random samples from 121 women who ranged in age from 17-39 years. The samples were selected from among a total of 3000 specimens included in a rheumatic disease investigation. Historic data on each of these women contained specific information regarding the use of OC. None of the 11 women with positive tests was using an OC.† Oral contraceptives in use by the women of Group 1A and Group 2 contained a synthetic estrogen and a progestagen. Tabulation of drug type, chemical composition, and the number of women using each, is listed in Table 2.

Serum was harvested from whole blood and divided into several aliquots which were stored at −20°C without added preservative. Each specimen was thawed only once before the several laboratory determinations. Serial serum specimens from the same patient were tested simultaneously.

Antinuclear antibodies were detected using a modification of the indirect immunofluorescent technic described by Friou (7). Four-micron liver sections from 2-month-old female Swiss Webster mice were prepared daily in a cryostat and fixed in 100% acetone on uncoated microscope slides. Fluorescein-conjugated horse antihuman globulin‡ was employed. Adsorption of the antiglobulin with mouse-liver powder did not alter the specificity of its reactivity with positive or negative sera. Each serum specimen was tested at 1:1 and 1:10 serum dilution. A serum specimen was considered positive if it was reactive at 1:10 serum dilution. Positive and negative control sera from the same individuals were used throughout this study.

Serum specimens were surveyed for rheumatoid factor activity using a latex-agglutination slide test.§ All sera that were positive or weakly reactive on slide test, as well as serum specimens from all women with positive tests for ANA were reassayed for rheumatoid factor by the latex tube-test of Singer and Plotz (8). Tests for RF were considered positive if they were reactive at a serum dilution of 1:40 or greater in the latex tube-test. C-reactive protein was measured by a capillary precipitation method using commercial antisera.‖ Duplicate capillary tubes containing equal volumes of patients'

*Initial follow-up interval varied between 1 and 12 months after starting OC and was: 1 month in 4 women, two months in 10, three months in 34, four to 6 months in 27, seven months in 2, eight months in 3 and 10–12 months in 2. In 9 women, three or more specimens were available at approximately 3, 6 and 9 or 12 months after starting OC.

†The women in Group 3 were representative of the general population. Sera from these women has been collected as part of the Tecumseh Health Study. This comprehensive community study included a special investigation of rheumatic disease (6).

‡Progressive Laboratories, Baltimore, Md.

§Hyland Laboratories, Costa Mesa, Calif.

‖Behringwerke, Woodbury, NY.

Table 1. Characterization of Study Groups

Group	No. of patients	Age		Months of OC use		White race
		Mean	Range	Range	Mean	
1. Never on OC*	174	20.9	16–40	0	0	90%
Follow-up on OC	82	20.9	18–35	1–12	4	95%
Follow-up not available	92	20.9	16–40			85%
2. On OC at time of initial exam*	210	24.9	17–47	3–84	28	85%
3. Community study	119†		17–39			100%

* Birth control clinic

† Two of the total number of 121 were excluded due to a positive test for ANA and concurrent use of OC

Table 2. Oral Contraceptives Taken by Study Patients

Composition	Proprietary name	Group 1A	Group 2
Combination			
norethindrone, 2 mg and mestranol, 0.1 mg	Ortho-Novum 2 mg Norinyl	7	51
norethindrone, 1 mg and mestranol, 0.05 mg	Ortho-Novum 1 mg	3	13
norethindrone, 1 mg and mestranol, 0.08 mg	Ortho-Novum 1/80	25	35
norethindrone acetate, 1 mg and ethinyl estradiol, 0.05 mg	Norlestrin 1 mg	2	6
norethynodrel, 2.5 mg and mestranol, 0.10 mg	Enovid E	1	7
ethynodiol diacetate and mestranol, 0.10 mg	Ovulen	15	48
norgestrel, 0.5 mg and ethinyl estradiol, 0.05 mg	Ovral	29	16
Sequential			
chlormadinone acetate 2 mg mestranol 0.08 mg	C-Quens	0	16
Other oral contraceptives*		0	11

* Group 2—Combination drugs: nonrethynodrel 2.5 mg, mestranol 0.075 mg (Enovid 5 mg) -4; medroxy progesterone 10 mg, ethinyl estradiol 0.05 mg (Norlutin)-1; medroxyprogesterone 10.0 mg, ethinyl estradiol .05 mg (Provest)-1;—Sequential Drugs: norethindrone 2 mg, mestranol 0.08 mg, (Ortho-Novum SQ)-4; dimethesterone 25 mg, ethinyl estradiol 0.1 mg (Oracon)-1.

serum and 5% albumin-saline were included to differentiate between cryoprecipitation and the presence of CRP. Tests for LE cell were performed on serum by a two-stage indirect method (9).

Immunoglobulin levels were assayed by radial immunodiffusion (10). Monovalent antisera to human IgG was prepared in this laboratory as described by Fahey (11). The antisera raised in rabbits was assayed for purity by immunoelectrophoresis and immunodiffusion. Test serum diluted 1:40 was applied to immunodiffusion plates containing 2% antiserum in 1% agar. Immunodiffusion plates for IgA and IgM were of commercial origin.* Standardized human serum,* containing known

*Behringwerke, Woodbury, NY.

concentrations of IgG, IgA and IgM, was assayed at three dilutions on each immunodiffusion plate. Normal range of values for adults 18–43 years of age, established in this laboratory are: IgG, 7.0–16.0; IgM, 0.5–2.3; IgA, 0.9–2.6 mg/ml.

The statistical analysis of these data employed the chi-square test for comparison of different groups of women (12). The probability values calculated in prospective studies on the same women, were obtained by comparing correlated percentages and from the Q test, a special chi-square test for matched samples (13).

RESULTS

Eighty-two of 174 Group 1 women who had not previously taken OC returned and donated a second blood specimen 1–12 months after starting these drugs. The results of the prospective studies in this group of women (Group 1A) are presented in Table 3. Seven women had positive tests for ANA before therapy and all remained positive during drug use. Four additional women with negative ANA tests prior to treatment developed positive tests for ANA after 1, 2, 7 and 8 months, respectively. It is of interest that these 4 women were taking oral contraceptive combinations that contained at least 80 μgm of Mestranol per tablet,* however, this was not a statistically significant association. Rheumatoid factor was detected in the serum of 3 women before, and in 9 more women after institution of OC. In these rheumatoid factor converters, no single drug combination predominated (Ovulin 21, 2; Ortho-novum 1/80, 4; Ovral, 2; Norlestrin, 1). They had, as a group, used oral contraceptives for a longer period (mean 6 months) than did the women who remained seronegative (mean 4 months). The titer of RF did not change in the 3 women who were positive before starting OC. C-reactive protein first appeared in the serum of 30 patients during use of OC, while 3 of 12 patients with positive tests before therapy became negative during treatment. LE cell phenomenon was not detected in any patient. Serologic conversion of each of these laboratory tests during the use of OC was significant ($P<0.02$ or 0.01).

Table 3. Serologic Abnormalities in 82 Women (Group 1A) Before and During Use of Oral Contraceptives

Serologic test	Before OC No.	Before OC % positive	On OC No.	On OC % positive	P
ANA*	7	8.5	11	13.4	<0.02
RF†	3	3.7	12	14.6	<0.01
CRP	12	14.6	39	47.6	<0.01
LE cell	0	0	0	0	

* 1:10 serum dilution
† 1:40 serum dilution

In the group of 82 women who were studied prospectively, the occurrence of one serologic abnormality increased the tendency for the occurrence of additional serologic abnormalities (Table 4). Seventeen of the 82 women accounted for all positive tests for ANA or RF that were found during treatment with OC. In these women, the prevalence of positive tests for CRP was much greater (70%) than that seen in seronegative Group 1A women (24 of 64 or 37%). Serum concentrations of IgG, IgM and IgA in seropositive women were measured before and during use of OC (Table 4). A moderate elevation in serum IgM level was found in 5 women before treatment and in 2 others during treatment with OC. Three of the 4 patients who developed ANA had increased serum concentrations of IgM before drug use. These elevations were not sustained during drug administration. Four of the 9 women who developed positive tests for RF had

*Ortho Novum (3), Ortho Pharmaceutical Corp., Raritan, NY and Ovulen 21 (1), G. D. Searle & Co, Chicago, Ill.

Table 4. Serologic Abnormalities (Group 1A) Before and During Use of Oral Contraceptives

	ANA*		RF†		CRP		IgG (mg/ml)		IgM (mg/ml)		IgA (mg/ml)	
Age	Pre	On	Pre	On	Pre	On	Pre	On	Pre	On	Pre	On
4 Seronegative women developed ANA												
19	0	+	0	1280	0	+	11.2	9.9	0.9	2.0	2.0	1.7
20	0	+	0	640	0	+	7.3	7.8	4.0	3.6	1.2	1.1
26	0	+	0	0	+	+	13.8	12.6	2.7	2.0	2.6	2.3
35	0	+	0	0	+	+	12.0	11.0	2.6	2.0	1.2	1.6
5 Seronegative women developed RF												
20	0	0	0	320	0	+	9.5	9.7	1.3	1.6	1.5	1.5
20	0	0	0	40	0	+	6.8	9.5	1.8	1.9	1.0	1.1
21	0	0	0	40	0	0	8.1	9.1	1.5	2.4	1.0	1.0
23	0	0	0	1280	0	+	9.0	9.1	1.9	2.2	1.7	1.6
25	0	0	0	640	0	0	11.0	11.0	1.6	1.4	2.8	2.6
7 women ANA positive before and during OC use												
18	+	+	1280	1280	0	+	11.9	10.3	1.5	1.3	1.0	1.9
19	+	+	0	0	0	0	9.2	8.8	2.0	1.6	3.3	2.6
20	+	+	2560	2560	+	+	10.6	9.3	3.0	4.3	2.1	1.4
20	+	+	0	640	+	0	8.2	8.0	2.0	2.3	0.9	1.0
20	+	+	0	0	0	+	7.0	6.9	2.2	1.9	1.8	1.4
21	+	+	0	320	+	+	11.2	10.8	3.0	3.0	1.1	1.0
28	+	+	0	0	+	+	15.4	10.4	1.7	1.7	2.0	1.5
1 woman RF positive before and during OC use												
22	0	0	640	640	+	+	6.7	6.5	1.3	1.0	1.5	1.2

* ANA positive at 1:10 serum dilution
† RF reciprocal titer

increased serum IgM levels during drug therapy. No consistent alteration in serum IgA concentration was noted. A mean decrease in serum IgG concentration from 10.0 ± 0.60 (mg/ml ± SEM) to 9.5 ± 0.51 occurred in seropositive women during treatment with OC. By contrast, in the 5 women whose only abnormality during treatment was the detection of RF, mean serum concentration of IgG increased from 8.8 ± 0.69 to 9.6 ± 0.33. Alterations in mean IgG serum concentration were compared with that observed in 12 seronegative women from Group 1A, matched for age and drug use with the seropositive group. The mean serum IgG level in the seronegative women was initially significantly lower (7.6 ± 0.46) than in the seropositive women, and decreased slightly during OC use to 7.3 ± 0.43 mg/ml. The observed alterations in immunoglobulin concentration did not correlate with positive tests for CRP.

In Table 5, the mean concentrations of IgG, IgM and IgA in the 17 seropositive women of Group 1A before and during OC treatment is summarized and compared with the findings in the 35 women of Group 2 who had positive tests for ANA or RF while taking OC. None of the seropositive women in Group 1A or Group 2 had an overt form of dysgammaglobulinemia. Six seropositive women of Group 2 had modestly increased IgM concentrations while taking OC (>2.3 mg/ml). None of the

Table 5. Immunoglobulin Levels in Sera of Women Positive for ANA or RF

Group	Observed (mean ± SE*)	Number of women: Within normal range†	Below normal range	Above normal range
1A pre-OC (17 of 82)				
IgG	10.0 ± 0.60	15	2	0
IgM	2.1 ± 0.20	12	0	5
IgA	1.6 ± 0.18	15	0	2
1A on OC (17 of 82)				
IgG	9.4 ± 0.38	15	2	0
IgM	2.2 ± 0.21	12	0	5
IgA	1.6 ± 0.14	17	0	0
2 on OC (35 of 210)				
IgG	10.0 ± 0.34	34	1	0
IgM	1.7 ± 0.11	29	0	6
IgA	1.8 ± 0.11	30	2	3

* Observed mean (mg/ml) ± standard error of the mean for all seropositive women

† Normal range of values for immunoglobulins in adults (18–43 years of age) as established in this laboratory are: IgG 7.0–16.0; IgM 0.5–2.3; IgA 0.9–2.6 mg/ml

women in Group 1A or Group 2 developed specific rheumatic complaints during treatment with OC.

In Table 6, the prevalence of all positive tests for ANA in Group 1 and in Group 2 is compared by chronologic age increments with women in Group 3. This latter group, matched for age, was selected at random from a total of 3000 serum specimens collected as a part of a community health survey. This study was required since the prevalence of positive tests for ANA in women 15–45 years of age has not previously been reported. Cross-comparison of the three groups failed to demonstrate a statistically significant difference in the prevalence of positive tests for ANA. The data does suggest that the frequency of positive tests for ANA increases with chronologic age.

When all pretreatment sera from Group 1 are compared with on-treatment sera from Group 2 women, the percentage of positive tests for ANA, RF and CRP is greater in Group 2 women (Table 7). When group comparisons were made, only the increase in positive tests for C-reactive protein was statistically significant ($P<.05$). Women in Group 2 had taken OC for longer periods of time (mean 28 months) than had women in Group 1A (mean 4 months). In Group 2 females, age, drug type or duration of use of drug could not be correlated with the occurrence of positive tests for ANA, RF or CRP. In addition, the titers of RF in Group 2 women were comparable to those observed in individual patients of Group 1 (1:40–1:2560). No one pattern of nuclear fluorescence predominated in any group. The pretreatment prevalence of ANA in Group 1 women was the same as that observed in a subgroup of 29 Group 3 women with the same mean chronologic age (20.9 years). Although Group 2 women had a higher number of positive tests for ANA (10%) than a subgroup of 24 women from Group 3 (4.2%) with the same mean age (24.9 years), the difference was not statistically significant. These results emphase the importance of the prospective data obtained during the Study of Group 1A women, in whom a statistically significant number of positive tests for ANA, RF and CRP developed.

DISCUSSION

Several drugs will induce autoantibodies in humans (14). The prospect that synthetic estrogens or progestagens found in oral contraceptives might induce similar serologic changes has been suggested by two recent reports. Schleicher (2) noted disap-

Table 6. Prevalence of Positive Tests for ANA by Age in Group 1, 2, 3

Age (yr)	Group 1, never used OC		Group 2, using OC		Group 3	
	%	No. positive/ tested	%	No. positive/ tested	%	No. positive/ tested
17–19	5.3	3/56	4.7	1/21	5.8	1/17
20–22	7.4	6/81	4.5	3/66	5.5	1/18
23–25	4.1	1/24	14.2	6/42	6.6	1/15
26–28	20.0	1/5	19.3	6/31	0	0/15
29–31	0	0/0	21.0	4/19	12.5	2/16
32–34	0	0/0	12.5	1/8	13.3	2/15
35–37	0	0/2	0	0/13	13.3	2/15
38–39	0	0/1	0	0/0	25.0	2/8
TOTAL POSITIVE/TOTAL TESTED		11/174		21/210		11/119

pearance of LE cell phenomena from the sera of 10 women after withdrawal of oral contraceptives. Bole (3) reported 8 women with early rheumatic complaints who had positive LE cell and/or ANA tests while taking oral contraceptives. When the patients stopped these drugs, LE cell phenomena and ANA frequently disappeared from their sera and rheumatic symptoms diminished. Spiera and Plotz (15) noted a relationship between the use of OC and the presence of rheumatic symptoms in 22 women. They did not detect any serologic abnormalities in these women during use of OC. Pretreatment sera were not examined by Schleicher or Bole, therefore, induction of autoantibodies was inferred from the disappearance of serologic abnormalities after withdrawal of OC. These observations concerned women presenting to the physician with early rheumatic complaints and gave no information as to the frequency of occurrence of abnormal tests for ANA, RF or LE phenomenon in asymptomatic women who were taking OC.

Table 7. Comparison of the Prevalence of Positive Serologic Tests Between Women of Groups 1, 2 and 3

Serologic test	Group 1 never OC		Group 2 on OC		Group 3		
	No.	%	No.	%	No.	%	*P*
ANA	11	(6.3)			2*	(6.9)	NS
			21	(10.0)	1†	(4.2)	NS
RF	8	(4.6)	16	(7.6)			NS
CRP	25	(14.4)	49	(23.3)			<0.05
Total	174		210				

* Two of a subgroup of 29 Group 3 women with same mean age as Group 1

† One of a subgroup of 24 Group 3 women with same mean age as Group 2

As discussed prevously, two preliminary reports (4, 5) that dealt with the occurrence of positive tests for ANA, LE cells or RF in apparently healthy young women while they were receiving OC have given disparate results. Another study (16) describes the development of rheumatic signs and symptoms in a large birth control clinic population during the use of oral contraceptives. In this series of 3014 women (90% black race), the incidence of rheumatoid arthritis (definite, probable, possible) increased from 9 per 3014 to 20 per 3014 over a mean period of 12 months of oral contraceptive use. Based strictly upon the low prevalence of rheumatoid arthritis

in OC users at the end of the study, the author concluded that OC did not influence rheumatic complaints. However, compared to the age-dependent increase in prevalence of rheumatoid arthritis reported in other population surveys (6, 17), this rate far exceeds the annual increment attributed solely to patient age. Detailed serologic studies on this group of women were not reported. It was noted that 2 of the 7 women with sickle cell anemia had positive tests for rheumatoid factor while taking OC.

Population and family studies have demonstrated that a small percentage of normal women will have autoantibodies (18), rheumatoid factor (19), and ANA (20), in the absence of rheumatic disease. In the Tecumseh Community Health Study (6), (the population from which Group 3 of this study was selected), 2% of women 20–29 years of age had rheumatoid factor detected by latex tube-test. This figure increased in 3.8% for women 30–39 years of age. Depending upon the methods employed for detection, 0–25% of normal women may have ANA. Svec (21) has recently reported an increase in the prevalence of these autoantibodies in women over 60 years of age. The value reached 25% in women past the age of 60. In the current study, an established, sensitive method for the detection of ANA demonstrated that the prevalence of positive tests increased in women between 17 and 39 years of age. Only in Group 1A women (mean age 21 years), where their pretreatment serologic status was known, and OC treatment interval short, could a significant association between OC use and serologic abnormalities be established. It is important to note that the prevalence of abnormal serologic tests in Group 1 women prior to OC treatment was higher than that reported in other population studies: 6% had ANA, 4% RF, and 14% CRP. It is also of interest that 4 of 11 serologic converters had pretreatment elevations of serum IgM levels as did 5 of the 8 women previously reported by Bole (3). However, no overt example of dysgammaglobulinemia was identified in any of these women. These findings suggest that apparently healthy young women reporting to a birth control clinic for contraceptive advice are not necessarily representative of all women in the child bearing years.

The composition and dose of the oral contraceptive may have influenced the induction of antinuclear antibodies. All 4 ANA converters were using a combination estrogen-progestagen that contained at least 80 μg Mestranol per tablet. This synthetic estrogen in similar or high dosage was also used by the symptomatic patients with positive tests for ANA previously reported by Bole and associates (3). In contrast to the preliminary observation of McKenna (5) that positive tests for RF correlated with the use of a Norethindrone-Mestranol combination, RF converters in the current study had used several different drug combinations. Current trends are toward use of oral contraceptives containing smaller amounts of synthetic estrogen in view of the statistical correlation of high estrogen content with thromboembolic disease (22). Although few women were studied, note should be taken of Dubois' observation (4) that RF or ANA was not detected in 8 women who used an OC containing only progestogen (chlormadinone).

CRP has been reported to appear in the serum of 72% of normal women when they use OC (23). In the current study, the high frequency of CRP induction permitted statistically significant conclusions to be drawn from group comparisons (Group 1 and Group 2) as well as from prospective

study of individual patients (Group 1A). There are major statistical limitations imposed on group comparisons when an event occurs infrequently (24). By contrast, prospective study of 82 women before and during oral contraceptive therapy allowed identification of 4 who developed ANA, 9 who developed rheumatoid factor, and 30 who developed CRP within one year after starting these drugs, and these associations were found to be significant.

None of the women studied before or during OC use developed symptoms of rheumatic disease. Long-term follow-up will be required to determine if women with autoantibodies in their serum initially or subsequent to oral contraceptive therapy have latent or subclinical autoimmune disease. Dissociation of serologic abnormalities from the observed presence of clinical disease is well recognized (25). Induction of serologic abnormalities in healthy young women who are taking OC should also be viewed from this perspective. However, recognition that oral contraceptives may cause positive reactions for ANA, RF or CRP is important to the proper use of these tests in clinical differential diagnosis of rheumatic disease.

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ADDENDUM

Participants at the April 16 and 17, 1970 Conference on Polymyalgia Rheumatica and Giant Cell Arteritis, held at the Mason Clinic, Seattle, Washington, and referred to in *Healey LA, Parker F, Wilske KR: Polymyalgia Rheumatica and Giant Cell Arteritis. Arthritis Rheum 14: 138–141, 1971,* included: Drs. H. Spiera, C. Plotz and S. Davison of New York; L. Fernandez-Herlihy, Boston; R. Reinecke, Albany; H. Kleinfelter, Baltimore; J. Decker and L. Sokoloff of Bethesda; K. Keller, New Orleans; C. Pearson, Los Angeles; W. Fessel, San Francisco; G. Missen, London; K. Wilske, F. Parker, M. Mannik and L. Healey of Seattle.