

EFFECTS OF ORAL CONTRACEPTION ON LIVER FUNCTION TESTS
AND SERUM PROTEINS IN WOMEN WITH PAST VIRAL HEPATITIS

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ABSTRACT

Forty-three women who had had viral hepatitis one or more years ago and 35 healthy women who were age and parity matched were given an oral contraceptive containing 0.05mg ethinyl estradiol and 0.5mg levonorgestrel for six consecutive months. Liver function tests (serum bilirubin, SGOT, SGPT and serum alkaline phosphatase) and serum proteins (total, albumin, globulins, ceruloplasmin, haptoglobin and alpha-1 antitrypsin) were measured before beginning treatment and after three and six months of use. Past hepatitis women experienced increased unconjugated bilirubin, SGOT, SGPT and alkaline phosphatase levels throughout the six months while the control women showed less pronounced changes during the first three months with tendency to reversion to normal during the subsequent three months; the group X time of test interactions were significantly different between the two groups. Serum haptoglobin decreased significantly in both groups but the past-hepatitis group showed a more persistent change with time. Changes also occurred in serum albumin, alpha-1 and beta globulins, ceruloplasmin but without group effect or group X time interactions.

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INTRODUCTION

Viral hepatitis is a prevalent infection in Egypt and in other developing countries. Twenty percent of the 13251 patients admitted to the Fever Hospital of Assiut during the year 1981 were having viral hepatitis. The hospital is draining a population of about 500,000. However, since notification is not compulsory, a large number of hepatitis cases, probably the majority, are treated outside the hospital. Consequently, a past history of viral hepatitis is present in many potential pill users and they are usually not denied such use. It is possible that these patients may have clinically undetected liver damage that may be adversely affected by the contraceptive steroids.

The present study was designed to investigate the effects of the use of a contraceptive pill on the liver function tests (LFTs) and on the serum levels of proteins produced in the liver in women with past-viral hepatitis. Specifically, the aim of the study was to determine if these women stand a higher risk of liver dysfunction while using the pill as compared to a matched control group of women having no history of previous liver disease.

MATERIAL AND METHODS

Women who had been discharged from the Assiut Fever Hospital one or more years ago after having been cured of viral hepatitis were called back for follow-up medical assessment. This comprized a physical examination and a battery of clinical laboratory tests including hemoglobin estimation, urinalysis, and measurement of LFTs: serum bilirubin, SGOT, SGPT, alkaline phosphatase, and of serum proteins: total, albumin, globulins, ceruloplasmin, haptoglobin, and alpha-1 antitrypsin. Of those who had normal LFTs and who expressed a desire to use the contraceptive pill and met the qualifying prerequisites, 89 women volunteered for the study. A similar number of healthy women who gave no past history of liver disease and who matched with women in the first group in age and parity were also recruited. Both groups were provided with an oral contraceptive containing 0.05mg ethinyl estradiol and 0.5mg levonorgestrel (Primovlar, Schering) for six consecutive months. The physical examination and the same battery of laboratory tests were repeated after three and six months of treatment. Over 75% of the patients and the controls were from rural areas. Giving repeated blood samples is so objectionable with women from this social background that only 43 women in the past-hepatitis group and 35 controls conformed to the study protocol and only their data will be presented. Their mean age was 30.3 years, and their mean parity was 4.3 children. Eighty-six percent of the women reported having used no contraceptives prior to enrolling in the study.

The methods used for the clinical laboratory tests are listed in Table I.

Table I. Methods Used for Clinical Laboratory Tests

Test	Reference for the method used
S. Bilirubin	Jendrassik, L. and Grof, P.: Biochem. Z, 297: 81, 1938.
SGOT and SGPT	Reitman, S. and Frankel, S.: Am. J. Clin. Path., 28: 56, 1957.
S. Alkaline Phosphatase	Bessey et al.: J. Biol. Chem., 164: 321, 1946.
S. Total Proteins	Miller, G.L.: Analyt. Chem., 31: 964, 1959.
S. Albumins and Globulins (electrophoresis on cellulose acetate strips)	Kohn, J. Chromatographic and Electrophoretic Techniques, Vol. II. Ed. Smith, I. William Heinemann Medical Books, Ltd., London, P., 56, 1960.
S. Ceruloplasmin) S. Haptoglobin) S. Alpha-1 Antitrypsin)	Single Radial Immunodiffusion Plates of Behringwerke AG, Marburg, W. Germany.

RESULTS

There was no difference between the past-hepatitis and control subjects in the incidence of any change in menstruation after pill use. At the time of admission to the study, there was no difference between the mean hemoglobin concentration of the past-hepatitis patients and that of the controls (11.1 ± 1.3 (S.D.)gm% and 11.2 ± 1.6gm%, respectively) and there was no change in these means along its course. There were no changes in blood pressure or weight over the course of the study.

Table II presents the mean values for each of the LFTs and serum proteins at admission and at each follow-up for both the control and past-hepatitis groups, while Table III shows the percentage changes from admission to each follow-up. Table IV depicts the number of subjects that showed, at the follow-ups, deviations outside the normal ranges. The latter are taken as two standard deviations on either side of the mean admission values for the control subjects. The highest incidence of such deviant results was noted for serum ceruloplasmin, followed by the transaminases, then serum bilirubin. The past-hepatitis women showed higher incidence of deviations in most of the variables as compared to the controls.

Repeated measures analyses of variance, with group (hepatitis vs control) as a between-subject variable and time of the test (admission, 3-month follow-up and 6-month follow-up) as a within-subjects variable, gave the following results:

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1. The changes in the LFTs were more pronounced in the past-hepatitis group than the controls. Moreover, the controls showed tendency to normalization of the changes during the fourth through the sixth month of treatment. Consequently, the group X time of test interactions were significant for serum bilirubin ($F(2,152) = 5.18, p < .01$), SGOT ($F(2,152) = 17.88, p < .001$), SGPT ($F(2,152) = 7.91, p < .001$) and alkaline phosphatase ($F(2,152) = 8.08, p < .001$).
2. No significant main effect for group or group X time of test interactions were observed for either the total proteins, albumin or any of the globulin subfractions. However, significant main effects for time were observed for total proteins ($F(2,152) = 26.00, p < .001$), albumin ($F(2,152) = 43.54, p < .001$), alpha-1 globulin ($F(2,152) = 12.22, p < .001$), and beta globulin ($F(2,152) = 7.70, p < .001$).

Table II. Mean Values of LFTs and Serum Proteins for Past-Viral Hepatitis and Control Groups at Admission, 3-Month Follow-up and 6-Month Follow-up

	Controls (n=35)			Past-Viral Hepatitis (n=43)		
	Adm	3-Mos	6-Mos	Adm	3-Mos	6-Mos
Serum Bilirubin						
Total (mg/100ml)	.48	.51	.47	.50	.67	.77
Conjugated	.17	.19	.17	.24	.29	.34
Unconjugated	.31	.33	.31	.26	.38	.44
Transaminases						
SGOT (U/L)	13.60	16.03	14.74	16.16	24.70	27.49
SGPT (U/L)	6.89	9.51	8.09	8.79	12.30	13.95
Alkaline Phosphatase (U/L)	28.71	30.57	29.09	28.58	34.30	33.40
Serum Proteins						
Total (gm/100ml)	6.93	6.57	6.68	6.90	6.23	6.44
Albumin	3.53	3.17	3.32	3.50	2.88	3.08
Globulins (Electrophoresis)	3.40	3.41	3.36	3.40	3.35	3.36
Alpha-1 Globulin	.34	.37	.41	.30	.34	.38
Alpha-2 Globulin	.67	.72	.72	.69	.69	.70
Beta Globulin	1.03	0.95	0.93	0.94	0.91	0.84
Gamma Globulin	1.37	1.36	1.30	1.47	1.41	1.45
Serum Ceruloplasmin (mg/100ml)	33.46	52.17	56.46	30.54	60.63	63.35
Serum Haptoglobin (mg/100)	141.11	109.74	122.03	152.67	128.93	119.26
Serum Alpha-1 Antitrypsin (mg/100ml)	264.00	332.29	357.43	186.58	250.56	295.67

3. Serum ceruloplasmin: A dramatic increase in ceruloplasmin levels was observed during the study ($F(2,152) = 129.18, p < .001$). Most of this increase occurred in the first three months. Neither the group main effect nor the group X time of test interaction reached significance.
4. Serum haptoglobin: A significant effect of time (admission >3-months = 6-months) and a significant group X time of test interaction ($F(2,152) = 9.10, p < .001$) were observed for this test. Although women in both groups had equivalent haptoglobin levels after six months, past-hepatitis patients experienced a steady decrease over the study period while levels for the control patients decreased more rapidly in the first 3 months and increased between 3 and 6 months.
5. Serum Alpha-1 antitrypsin: This was the only variable in which the two groups differed at admission, the past-hepatitis group had significantly lower serum alpha-1 antitrypsin which resulted in a significant effect for group ($F(1,76) = 21.50, p < .001$). In addition, the effect for time of test was significant ($F(2,152) = 76.74, p < .001$), with 6-month > 3-month > admission levels.

Table III. Percent Change, by Group, in LFT Between Admission and 3-Month Follow-up and Between Admission and 6-Month Follow-up

	<u>Control</u>		<u>Past-hepatitis</u>	
	Adm-3mos	Adm-6mos	Adm-3mos	Adm-6mos
	%	%	%	%
Serum Bilirubin				
Total	+ 6	- 2	+34	+54
Conjugated	+12	0	+21	+42
Unconjugated	+ 6	0	+46	+69
Transaminases				
SGOT	+18	+ 8	+53	+70
SGPT	+38	+17	+40	+59
Alkaline Phosphatase	+ 6	+ 1	+20	+17
Serum Proteins				
Total	- 5	- 4	-10	- 7
Albumin	-10	- 6	-18	-12
Globulin	0	- 1	- 1	- 1
Alpha-1	+ 9	+21	+13	+27
Alpha-2	+ 7	+ 7	0	+ 1
Beta	- 8	-10	- 3	-11
Gamma	- 1	- 5	- 4	- 1
Serum Ceruloplasmin	+71	+85	+99	+107
Serum Haptoglobin	-22	-14	-16	-22
Serum Alpha-1 Antitrypsin	+26	+35	+34	+58

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Table IV. Number of Women Showing Laboratory Test Values Out of Normal Range* at Each Follow-up

Test	Normal Range		Controls (n=35)		Past-hepatitis (n=43)	
			3-mos	6-mos	3-mos	6-mos
Serum bilirubin	0.16 -	0.80 mg/100 ml	4	1	15	17
Conjugated	0.00 -	0.34 mg/100 ml	1	0	13	16
Unconjugated	0.10 -	0.51 mg/100 ml	1	0	10	9
SGOT	6.1 -	21.1 U/L	5	3	22	25
SGPT	2.1 -	11.7 U/L	9	3	19	20
Alkaline Phosphatase	6.7 -	50.7 U/L	2	0	5	6
Total Proteins	5.4 -	8.5 gm/100 ml	2	2	8	9
Albumin	2.7 -	4.4 gm/100 ml	5	2	18	10
Globulin	2.5 -	4.3 gm/100 ml	1	1	1	4
Alpha-1 Globulin	0.10 -	0.58 gm/100 ml	3	4	2	4
Alpha-2 Globulin	0.37 -	0.96 gm/100 ml	0	0	4	1
Beta Globulin	0.71 -	1.34 gm/100 ml	6	9	8	14
Gamma Globulin	0.78 -	1.96 gm/100 ml	1	1	3	4
Ceruloplasmin	14.6 -	46.3 mg/100 ml	19	25	29	32
Haptoglobin	77.1 -	205.2 mg/100 ml	7	2	1	1
Alpha-1 Antitrypsin	114.6 -	413.4 mg/100 ml	5	8	4	4

* Normal range= two standard deviations on either side of mean of the admission values for the control healthy women.

Table V shows the correlation between the percentage changes after 6 months of pill use in serum ceruloplasmin, haptoglobin and alpha-1 antitrypsin with the percentage changes in tests usually interpreted as indicative of liver dysfunction. Increase in ceruloplasmin correlated significantly with increase in serum bilirubin and did not correlate with changes in total proteins, albumin, SGOT, SGPT or alkaline phosphatase. The changes in alpha-1 antitrypsin showed border-line ($r > .20$, $p < .05$) correlations with changes in serum bilirubin levels and SGPT activity. Changes in haptoglobin did not correlate significantly with changes in any of the LFTs.

Table V. Correlation* Between Changes in the Specific Proteins and Changes in LFTs After 6 Months of Pill Use

	Cerulo- plasmin	Haptoglobin	Alpha-1 Antitrypsin
Bilirbin	0.2760 (0.007)	-0.0017 (0.494)	0.2053 (0.036)
Total proteins	-0.1049 (0.180)	0.0864 (0.226)	-0.1050 (0.180)
Albumin	-0.0908 (0.215)	0.1819 (0.056)	-0.1274 (0.133)
Alkaline phospha- tase	0.1334 (0.122)	-0.0169 (0.442)	0.2263 (0.023)
SGOT	0.0151 (0.448)	-0.1613 (0.079)	0.0719 (0.266)
SGPT	0.1367 (0.116)	-0.1015 (0.188)	0.2055 (0.036)

* Correlation coefficient; significance in parentheses.

DISCUSSION

The use of a contraceptive pill containing 0.05mg ethinyl estradiol and 0.5mg levonorgesterel resulted in development of abnormalities in liver function in a small proportion of the healthy control women. The incidences of abnormalities, however, were generally higher than those reported for other populations (1-4). The differences are undoubtedly a function of the difference in the type of pill used and in the duration of use. Factors such as higher parity and the relatively defective nutritional standards in our subjects might also be

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contributing factors. However, as in previous studies, our control subjects showed a tendency toward normalization of LFTs on prolongation of pill use.

At admission to the study, the past-hepatitis women did not differ significantly from the controls in any of the LFTs. However, significant group difference developed after treatment in serum bilirubin, SGOT, SGPT and alkaline phosphatase. The incidences of abnormalities in the LFTs were greater in the past-hepatitis group than in the control, and the former group showed less tendency for improvement of tests after prolonged use. It seems that some women with clinical and biochemical evidences of cure of hepatitis are still having some residual abnormalities in their liver cells that will become biochemically detectable when exposed to contraceptive steroids. A small-scale study by Eisalo *et al.* (5) has, however, given different findings. Administration of oral contraception to 10 women with past viral hepatitis did not result in significant changes in LFT after two to three months.

Based on our results, we feel that a past history of viral hepatitis should indicate assessment of liver functions before pill use, and that the pill should not be used by women with abnormalities. For those with normal LFTs, a low-dose estrogen pill will be the better type prescribed. Repeat testing after a few months are required and may reveal subtle abnormalities that are unmasked by pill use.

In contrast, no significant main effect of group or group X time of test interactions were observed for either serum total proteins or any of protein subfractions studied. Collapsed across the two groups, there were, however, main effects of time of test for some serum proteins. The treatment resulted in a significant decrease in the concentration of total proteins, albumin, haptoglobin and beta-globulin and a significant increase in alpha-1 globulin and alpha-1 antitrypsin.

Ceruloplasmin is exclusively produced in the liver microsomes (6) and its serum level correlates with serum copper in women on oral contraceptives (7). Haptoglobin is actually a group of mucoproteins synthesized in the liver and belong to the alpha-2 globulin of the serum. They have the specific function of binding free hemoglobin liberated intravascularly and facilitate its elimination in the reticuloendothelial system (8). There are recent indications that, through this latter function, haptoglobin may act as a natural bacteriostat depriving adventitious bacteria from the support of hemoglobin iron for their proliferation (9). Low haptoglobin levels were reported in hepatocellular failure (8). The decrease in the concentration of haptoglobin following oral contraceptives has also been reported by Briggs and Briggs (10). Alpha-1 antitrypsin is a glycoprotein

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synthesized in the liver and it is the principal alpha-1 globulin in the serum. The increased levels of alpha-1 antitrypsin on pill use is similar to the findings of a previous report (11). In the present study the elevation observed in serum alpha-1 globulin from pill use differs from results of Musa et al. (12) who reported no significant change in this fraction.

The present study indicates that, with few exceptions, changes in ceruloplasmin, haptoglobin and alpha-1 antitrypsin do not correlate with changes in test results usually taken as indicative of liver dysfunction. The multiple changes in the serum levels of proteins produced in the liver do not necessarily indicate hepatic damage. They can result from altered rates of anabolism or catabolism. However, to dismiss these changes as harmless, when continued over many years encompassing the reproductive period of a woman's life, is open to question.

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