The effect of oral contraceptive therapy and of pregnancy on serum folate levels of rural Sri Lankan women

BY NAVAM S. HETTIARACHCHY,* SACHI S. SRI KANTHA† AND S. M. X. COREA

Department of Biochemistry and Department of Community Medicine, Faculty of Medicine, University of Peradeniya, Sri Lanka

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1. Serum folate level, packed cell volume and haemoglobin concentration of apparently healthy rural Sri Lankan women, between 20 and 45 years and earning up to Rs. 500 (Sri Lankan Rs. 35 ≃ £1 Sterling) per month, were estimated during pregnancy and when on oral contraceptive (OC) treatment with Ovulen 50 (ethinyl oestradiol 0·05 mg, ethinodiol diacetate 1 mg).

2. Ovulen 50 administration led to a fall in serum folate levels which occurred very rapidly during the first 6 months and more slowly thereafter, stabilizing at 2·2 ng/ml in women of very low economic status and at 2·9 ng/ml in the more privileged.

3. There was a steady fall in serum folate concentrations during pregnancy, the levels at the end of pregnancy being higher than those in women under OC treatment for 9 months.

4. The need for folate supplementation during OC treatment is stressed.

Published research studies indicate that the concentration of folic acid in blood serum is low in women on oral contraceptive (OC) therapy (Shojania *et al.* 1968, 1969, 1971; Ahmed *et al.* 1975). McLean *et al.* (1969) compared the serum folate levels of thirty-nine women receiving oral contraceptives at a clinic in rural north central Florida with that of seventeen controls and found no difference between the two groups. They concluded that sufficient folate was absorbed by those on OC therapy to maintain serum folate levels within normal limits. Castren & Rossi (1970), as well as Pritchard *et al.* (1971), could not find significant differences in serum folate levels between controls and those on OC therapy, or in women on OC therapy before and after stopping the contraceptive pill. Most of these studies, however, have been carried out on well-nourished women in industrialized countries. On the other hand, a report from India (Ahmed *et al.* 1975) indicated that prolonged OC therapy could seriously impair the folate status of women.

In popularity ranking of methods of contraception, oral contraceptives ranked fourth in Sri Lanka at the time of the World Fertility Survey in 1975. Of the 35% of married, fecund women who were using contraceptive methods in Sri Lanka, 5% were found to be users of oral contraceptives (Lightbourne & Singh, 1982). Furthermore, the evidence of nutritional inadequacy among the vulnerable age-groups in rural Sri Lanka has also been documented (Jogaratnam & de Mel, 1976). The present investigation was initiated to study (*a*) the serum folate levels of healthy non-pregnant women, (*b*) the effect of a combination type of OC (Ovulen 50; ethinyl oestradiol 0·05 mg, ethinodiol diacetate 1 mg) on serum folate levels, and (*c*) the effect of pregnancy on serum folate levels, among the Sri Lankan women.

Present addresses: * 11 Goodhill Road, New Milford, Connecticut 06776, USA. † Department of Food Science, 567 Bevier Hall, 905 S. Goodwin Avenue, University of Illinois, Urbana, Illinois 61801, USA.

MATERIALS AND METHODS

Subjects. Female subjects selected for the study were those attending the family planning clinic, Buwelikade, and the antenatal clinic, Kadugannawa, both in the Kandy district of Sri Lanka. Those who had received folate supplements or had given birth within 1 year of the commencement of the study, or had a history of recent illness or medication, were excluded. All subjects had a family income of less than Rs. 500 per month (35 Sri Lankan Rs. ≃ £1 Sterling) and were between 20 and 45 years of age.

Blood samples were drawn from the subjects at least 3 h after a light breakfast, when they reported for continuation of oral contraceptive, for insertion of intra-uterine contraceptive devices or for routine checks at the antenatal clinic.

Expt 1. Effect of OC therapy on serum folate levels

Cross-sectional study. Women (*n* 93) in this study were those who had been on OC therapy (Ovulen 50) for a period of 3–18 months.

Longitudinal study. Forty-six subjects considered healthy on clinical examination were selected for OC therapy. Blood samples were taken at the commencement of treatment and during the 3rd, 6th, 12th and 18th month of Ovulen 50 therapy.

Expt 2. Effect of pregnancy on serum folate levels

Cross-sectional study. Blood samples were taken from healthy pregnant women (*n* 172) during visits to the antenatal clinic. At the clinic, they were given supplements of calcium and iron but not of folate.

Longitudinal study. Fifty apparently normal women were studied during pregnancy and blood samples drawn at the end of the first, second and third trimesters. The women had not been on OC therapy before their pregnancy commenced.

Controls. Healthy women (*n* 184) attending the family planning clinic for insertion of intra-uterine contraceptive devices, served as controls for both experiments.

All subjects had been considered in three age-groups (years: group 1, 20–25; group 2, 26–35; group 3, 36–45) and at three income levels (Rs./family per month: level 1, 50 or less; level 2, 51–250; level 3, 251–500). At the commencement of the study, when it was found that the differences between income levels 2 and 3 were not significant, subjects were considered at two income levels only, those earning Rs. 50 or less and those earning more than Rs. 50 per month.

Estimations of packed cell volume (PCV), haemoglobin (Hb) and mean corpuscular Hb concentrations (MCHC)

PCV and Hb concentrations were estimated within 3 h of collection of blood samples; PCV by the micro-haematocrit method and Hb by the oxyhaemoglobin method. MCHC was calculated from the PCV and Hb concentrations.

Estimation of folate

Serum folate concentrations were determined by the radioisotopic protein-binding method described previously by Piyasena *et al.* (1977). All serum samples were stored at −20° with added ascorbic acid (5 mg/ml) and assayed within 2 weeks of collection. Serum samples of low, normal and high folate concentrations were pooled separately and used for the preparation of quality-control pools included in each assay run.

Statistical analyses

Values were compared using Student's *t* test for the cross-sectional and longitudinal studies

Table 1. *Expt 1. Cross-sectional study of packed cell volume (PCV), haemoglobin (Hb), mean corpuscular Hb concentration (MCHC) and serum folate measurements in control and oral contraceptive therapy (OC)-treated rural Sri Lankan women of different age-groups and income levels*

(Mean values with their standard errors; no. of subjects in parentheses)

		Income level (Rs./month)					
		1 (less than 50)		2 (from 51 to 250)		3 (from 251 to 500)	
		Control	OC-treated	Control	OC-treated	Control	OC-treated
	Age group†	Mean SE	Mean SE	Mean SE	Mean SE	Mean SE	Mean SE
PCV	1	0·414 0·009 (25)	0·415 0·009 (8)	0·426 0·006 (25)	0·442 0·01 (9)	0·422 0·009 (25)	0·437 0·012 (5)
	2	0·409 0·7 (20)	0·415 0·011 (6)	0·412 0·006 (20)	0·425 0·009 (16)	0·405 0·010 (20)	0·433 0·013 (9)
	3	0·390 0·004 (16)	0·425 0·009 (11)	0·399 0·006 (16)	0·438 0·006 (14)	0·418 0·007 (17)	0·440 0·009 (14)
Hb (g/l)	1	122 2 (25)	133 2 (8)	138 2 (25)	142 3 (9)	139 2 (25)	147 4 (5)
	2	133 1 (20)	134 3 (6)	136 1 (20)	143 2 (16)	138 1 (20)	141 2 (9)
	3	129 2 (16)	145 3 (11)	139 2 (16)	141 3 (14)	141 2 (17)	144 3 (14)
MCHC (g/l)	1	302 7 (25)	328 7 (8)	325 6 (25)	322 5 (9)	320 8 (25)	338 16 (8)
	2	327 6 (20)	323 7 (6)	332 6 (20)	338 10 (16)	328 7 (20)	328 14 (9)
	3	331 5 (16)	341 8 (11)	350 5 (16)	321 7 (14)	339 7 (17)	329 11 (14)
Serum folate (ng/ml)	1	4·0 0·2 (25)	2·8 0·1** (8)	4·8 0·3 (25)	2·9 0·2** (9)	4·9 0·3 (25)	3·6 0·3** (5)
	2	4·0 0·2 (20)	2·5 0·2** (6)	5·2 0·3 (20)	3·2 0·2** (16)	5·2 0·3 (20)	3·6 0·2** (9)
	3	4·0 0·3 (16)	2·8 0·1** (11)	5·1 0·3 (16)	3·2 0·2** (14)	5·2 0·3 (17)	2·8 0·2** (14)

† 1, 20–25 years; 2, 26–35 years; 3, 36–45 years.
Values for OC-treated subjects were significantly different from those of the corresponding controls: ** $P < 0·01$.

of Expt 1, and for the cross-sectional study of Expt 2. Paired t tests were used for the longitudinal study of Expt 2 (Snedecor & Cochran, 1980).

RESULTS

Expt 1

The cross-sectional survey of haematological measurements and serum folate levels of 184 control subjects and ninety-two subjects on OC treatment is provided in Table 1. A ıong the controls, the mean Hb and serum folate concentrations at the lowest income level of Rs. 50 per month or less (level 1) were significantly lower ($P < 0·001$) than the mean values of those earning more than Rs. 50 per month (levels 2 and 3). Subnormal serum folate levels of lessl than 3·0 ng/ml were found in 12% of women at income level 1, 7% of those at level 2 and 7% of those at level 3.

The mean (with SE) serum folate level of the subjects on OC therapy was found to be

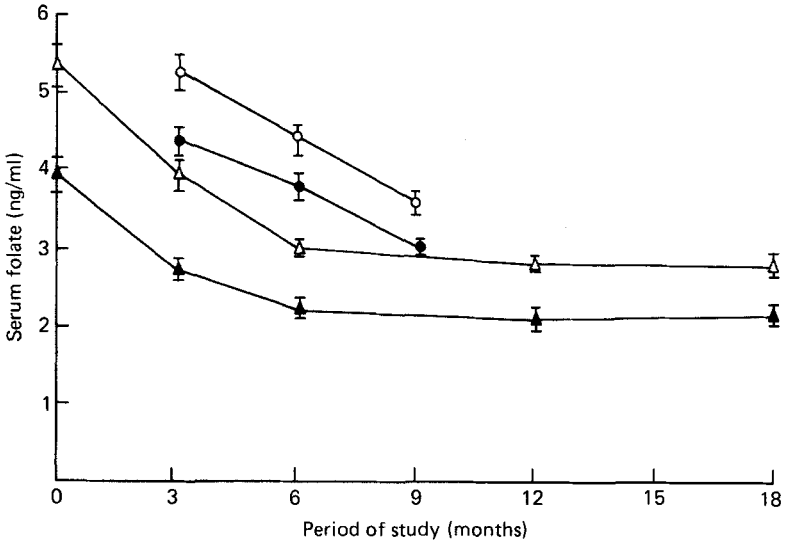

Fig. 1. Change in level of serum folate during pregnancy and during oral contraceptive therapy in rural Sri Lankan women. (○) Pregnancy, higher income level (Rs. 51–500 per month); (●) pregnancy, lower income level (less than Rs. 50 per month); (△) oral contraceptive therapy, higher income level; (▲) oral contraceptive therapy, lower income level.

significantly lower ($P < 0{\cdot}001$) than the level of 4·7 (0·1) ng/ml present in the control subjects. Furthermore, within the OC treatment group, the mean folate level (2·7 (0·1) ng/ml) in the women of income level 1 was also significantly lower ($P < 0{\cdot}01$) than the mean (3·2 (0·1) ng/ml) value of those of income levels 2 and 3. It was also evident that the mean serum folate value of 2·7 (0·1) ng/ml of the OC-treated low-income group of women differed significantly from the mean serum folate value of 4·0 (0·1) ng/ml of the control subjects in income level 1. Among the OC-treated subjects, 64% at income level 1 and 33% of those at income levels 2 and 3 had serum folate concentrations of less than 3·0 ng/ml.

Fig. 1 shows the results obtained in the longitudinal study on the effect of OC on serum folate levels. A steady decline in the folate concentrations was observed in all age-groups. At both the lower and the higher income levels, the serum folate concentrations in pregnant women were greater than those of women on OC therapy. The folate levels in the OC-treated subjects appeared to stabilize after the 12th month of treatment at 2·2 ng/mg in the lower-income group, and at 2·9 ng/ml in the higher-income group. However, other haematological factors such as PCV and Hb levels were found to remain fairly constant during the 18-month OC therapy.

Expt 2

Of the 172 women who volunteered for the cross-sectional study of the effect of pregnancy on serum folate levels, eighty-seven received an income of Rs. 50 or less, and eighty-five an income of more than Rs. 50. Of the fifty longitudinal-study subjects, twenty-three were in the low-income group (level 1) and twenty-seven in the higher income group (levels 2 and 3).

Table 2 shows the changes in the levels of serum folate with advancing stages of pregnancy. In the cross-sectional study, the serum folate concentrations of women of income level 1 fell from a mean (with SE) of 4·0 (0·1) ng/ml in the first trimester to 3·8 (0·1) and

Table 2. *Expt 2. Effect of pregnancy on serum folate levels of rural Sri Lankan women*
(Mean values with their standard errors; no. of subjects in parentheses)

Income level (Rs./month)	Trimester: First Mean	First SE	Second Mean	Second SE	Third Mean	Third SE
			(*a*) Cross-sectional study			
50 or less	4·0	0·1[a] (27)	3·8	0·1[a] (33)	3·4	0·1[b] (27)
More than 50	5·3	0·3[a] (28)	5·1	0·2[a] (29)	3·7	0·1[b] (28)
			(*b*) Longitudinal study			
50 or less	4·4	0·2[a] (23)	3·8	0·2[b] (23)	3·1	0·7[c] (23)
More than 50	5·2	0·2[a] (27)	4·4	0·2[b] (27)	3·6	0·1[c] (27)

[a, b, c] Values within the same horizontal row with different superscript letters were significantly different ($P < 0·01$).

3·4 (0·1) ng/ml in the second and third trimesters respectively. The difference between the concentrations in the second and third trimesters was significant ($P < 0·01$) and so was the overall drop between the first and third trimesters ($P < 0·01$). Similar results were seen also for women at the higher income levels (more than Rs. 50 per month). In the longitudinal study, at both low and higher income levels, the fall in serum folate concentrations as pregnancy progressed from the first to the third trimester was significant ($P < 0·001$).

In Table 3, the changes in Hb concentrations with advancing stages of pregnancy are shown. The variations seen were similar to the corresponding pattern exhibited by serum folate changes. There was a significant fall ($P < 0·01$) between the first and third trimesters, at both low and higher income levels in the cross-sectional study. Similarly, the change in Hb concentrations with advancing period of gestation in the longitudinal study was also significant ($P < 0·001$).

DISCUSSION

More than 50% of families in Sri Lanka earn less than Rs. 300 per month (Richards & Gooneratne, 1980), so that the women of income level 1 in the present study are representative of part of a majority of women in the country. The mean (with SE) serum folate level of 4·7 (0·1) ng/ml in the control subjects is much lower than the values (6·1 (2·2) ng/ml) reported for Canadian women (Shojania *et al.* 1971). The mean folate level of those Canadian women under OC therapy (4·2 (1·7) ng/ml) was also higher than the value of 3·2 (0·1) ng/ml seen in the present study for the higher-income groups.

The results indicate unequivocally that prolonged OC therapy (more than 12 months) leads to very low serum folate concentrations in the population studied. The levels were much lower than the levels reached at the end of pregnancy, in spite of the fact that during pregnancy the daily hormone production is greater than the hormones supplied by Ovulen 50 to the OC users, and that there is a continuous withdrawal of folate from the mother by the fetus. Pregnant women of the present study sample from the Kandy district are nutritionally a vulnerable group. This had been documented by other researchers (Gardner *et al.* 1977) in terms of the prevalence of a high incidence of anaemia seen among the female population of the hill country districts of Sri Lanka. Hence, the inference that pregnant women, as well as the OC users of the present study, were subject to depreciation in body folate levels is not without foundation. Furthermore, the relationship between the low economic status of the study sample and the depleted serum folate levels cannot be ignored.

Table 3. *Effect of pregnancy on haemoglobin levels of rural Sri Lankan women*
(Mean values with their standard errors; no. of subjects in parentheses)

Income level (Rs./month)	Trimester					
	First		Second		Third	
	Mean	SE	Mean	SE	Mean	SE
			(*a*) Cross-sectional study			
50 or less	13·2	0·1[a] (27)	12·9	0·2[a] (33)	12·1	0·1[b] (27)
More than 50	13·3	0·2[a] (28)	12·8	0·1[a] (29)	12·4	0·1[b] (28)
			(b) Longitudinal study			
50 or less	12·6	0·2[a] (23)	12·1	0·1[b] (23)	11·8	0·1[c] (23)
More than 50	13·0	0·1[a] (27)	12·2	0·1[b] (27)	11·8	0·1[c] (27)

[a, b, c] Values within the same horizontal row with different superscript letters were significantly different ($P < 0·01$).

Liver, an animal source of folate, was rarely consumed by the subjects due to its prohibitive cost inthe food market. Therefore, green leafy vegetables were the easily available rich source of folate for these subjects. However, the intake of green vegetables among the study sample was found to be irregular; improper cooking techniques and nutritional ignorance also contributed to inadequate intake of food folates, since very few of the subjects had known about the thermo-labile nature of this water-soluble vitamin.

Dietary folates largely consist of polyglutamates. The deconjugation step in the small intestine before absorption had been recognized as vital for adequate absorption to take place. Studies by Streiff (1970) suggest that this enzymic step is affected by OC drugs. Although OC therapy did not significantly change the absorption of monoglutamic folate, a 50% decrease in the absorption of polyglutamic folate had been observed (Streiff, 1970). However, a later study by Shojania & Hornaday (1973) failed to demonstrate polyglutamate malabsorption, and these workers have suggested the existence of some other mechanism which could be responsible for polyglutamate malabsorption. Another study (Paine *et al.* 1975) has indicated that polyglutamate malabsorption, if uniformly present, is seldom reflected by low serum folate values and is only rarely clinically important. However, all these studies had used women of comparatively high economic status from developed countries. Though conclusive confirmatory evidence is yet to be presented regarding the absence of an influence of OC on folate levels, the general consensus of opinion that an alteration in the folate metabolism during pregnancy could occur, with a fluctuation in the levels of female sex hormones, still remains unchallenged (Colman, 1977).

In conclusion it is suggested that, if women belonging to a low income group (similar to the present study sample) are encouraged to resort to OC therapy for family spacing, it is conceivable that, when they desire to have a child, they would commence pregnancy in a folate-depleted state, making the course of pregnancy more hazardous. In accordance with Streiff's (1970) proposal, it suggested that adequate supplementation with folate during pregnancy or combining folate with the hormones in the contraceptive pill, or both, seem to be advisable for the women of developing countries such as Sri Lanka.

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