Maternal Milk and Serum Vitamin B12, Folic Acid, and Protein Levels in Indian Subjects

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Jathar, V. S., Kamath, S. A., Parikh, M. N., Rege, D. V., and Satoskar, R. S. (1970). Archives of Disease in Childhood, 45, 236. Maternal milk and serum vitamin B12, folic acid, and protein levels in Indian subjects. Serum vitamin B12 and folic acid activity levels were studied in 47 mothers. The mean serum vitamin B12 value in non-vegetarian mothers was 228 μg./ml. ± 38.9 SE, as compared to mean values 97 μg./ml. ± 20.7 SE in lacto-vegetarians. There was no significant difference in folic acid levels, haemoglobin, and total proteins among the different dietetic groups. Analysis of milk revealed mean vitamin B12 values of 103 μg./ml. in non-vegetarians as compared to 91 μg./ml. in lacto-vegetarians; the difference was not statistically significant. Mean total protein and folic acid levels for milk were similar in different dietetic groups. The figures obtained for vitamin B12 content of human milk in these mothers are considerably lower than those reported in similar studies from Western countries.

In India, the dietary intake of animal protein food in the majority of the population is very low, either because of religious convictions or because of its high cost. The intake of milk or milk products is also low among the general population (Patwardhan, 1961; Antia, 1966). Previous studies have shown that the mean serum vitamin B12 level in apparently healthy lacto-vegetarian Indians is much lower than the mean observed in non-vegetarian subjects (Dhopheswarkar et al., 1956; Satoskar, Kulkarni, and Rege, 1961; Mehta, Rege, and Satoskar, 1964). During pregnancy the mean serum vitamin B12 usually shows a fall without any associated changes characteristic of B12 deficiency anaemia (Boger et al., 1957; Baker et al., 1958; Prystowsky et al., 1959; Baker et al., 1957; Young et al., 1959; Lowenstein et al., 1960; Metz, Festenstein, and Welch, 1965; Edelstein et al., 1966; Martin, Davis, and Stenhouse, 1967).

Infants receive their vitamin B12 and folic acid requirements mainly from mother's milk. In India, where baby food is only used by the affluent class, mothers' milk is the only source of nutrition for the majority of infants. Breast feeding is usually started within two or three days after delivery and, unlike Western countries, is continued into the second or even third year of life (Belavady, Pasricha, and Shankar, 1959; Gopalan and Belavady, 1961). This study was, therefore, undertaken to investigate the composition of breast milk with particular reference to its vitamin B12 content in Indian mothers with various dietetic habits. Simultaneously, folic acid levels were also studied. A preliminary account of the observations was reported earlier (Jathar et al., 1967).

Material and Method

The study was carried out on 48 mothers between the ages of 18 and 35 years admitted to the Nowrosji Wadia Maternity Hospital, Bombay, and on 65 healthy non-pregnant women of a similar age-group. All of them belonged to the low socio-economic group and thus represented the majority of the population. Their diet essentially consisted of varying amounts of rice, wheat, pulses, green vegetables, and rarely fruit. Only 17 mothers took some non-vegetarian food daily, while 15 mothers were strictly lacto-vegetarians; the remaining 16 took non-vegetarian food only occasionally. Even among the 'non-vegetarians' the daily intake of animal protein was low as compared to Western standards.

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Milk samples were obtained early in the morning before feeding the babies, in sterile conical flasks by manual expression, and blood was collected at the same time from an ante-cubital vein. Milk B12 was estimated microbiologically using Euglena gracilis var. bacillaris as the test organism. The test samples of milk were treated with papain and sodium cyanide solution (1% w/v) and were incubated under toluene at 37 °C. overnight. The extracts were filtered, and from the clear filtrate, a measured portion was analysed for its vitamin B12 content. Serum and milk vitamin B12 assays were carried out as described by Ross (1952).

For the estimation of folic acid activity (FAA), serum and milk samples were treated with 0.05 M sodium phosphate buffer, pH 6.1, and 150 mg/100 ml. ascorbic acid. The protein was separated out and filtrates were assayed using L. casei and S. faecalis R as test organisms by the method of Herbert (1961).

Total serum proteins were determined by the Biuret method. For the estimation of milk protein the total nitrogen was determined by micro-digestion followed by direct nesslerization and the values obtained were multiplied by 6.28. Haemoglobin and packed cell volume were estimated by standard techniques.

**Results**

Nearly 75% of the subjects took very little milk except perhaps with tea. The major protein source in the diet was of vegetable origin mainly in the form of pulses. Only 17 subjects had some fish, meat or eggs in their diet and these were classified as 'non-vegetarians—frequent meat eaters'.

The mean weight of these mothers was 45.4 kg., with a range of 36.0 to 57.0 kg.

**Serum composition.** Serum proteins, vitamin B12, and FAA in these subjects classified according to their diet are given in Table I. Both the mean haemoglobin and haematocrit values were higher in mothers who took non-vegetarian food at least once daily than in the lacto-vegetarian mothers whose major protein source was of vegetable origin. The differences, however, are not statistically significant. The serum vitamin B12 levels in lacto-vegetarians were much lower than those observed in the non-vegetarian group. Thus the mean serum vitamin B12 level in lacto-vegetarians was 97.3 μg/ml., as compared to 228.3 μg/ml. in non-vegetarians. Individuals who partook of non-vegetarian food occasionally had mean serum vitamin B12 values of 181.5 μg/ml. The mean serum proteins and FAA values, however, were similar in the three dietetic groups.

Serum vitamin B12 values observed in lactating Indian mothers are compared with apparently healthy non-pregnant Indian women belonging to a similar age-group in Table II. The mean serum vitamin B12 value in non-pregnant lacto-vegetarians was significantly lower than that observed in the corresponding non-vegetarian group. As compared with the non-pregnant group, both the haemoglobin and serum vitamin B12 values showed a fall in lactating mothers. Thus lacto-vegetarian mothers had mean serum vitamin B12 values of 97.3 μg/ml. as compared to 139.5 μg/ml. in normal subjects. The difference was statistically significant (p > 0.01). A similar difference was also observed in non-vegetarian lactating mothers. The distribution of vitamin B12 levels in various groups analysed according to diet is shown in the Figure.

There was no correlation between the serum vitamin B12 levels and haemoglobin values.

**Vitamin B12, FAA, and protein content of milk.** These are presented in Table III. Levels of vitamin B12 in breast milk for the whole group averaged 100.3 μg/ml. Analysis of milk in various dietetic groups revealed mean vitamin B12 values of 91.3 μg/ml. in lacto-vegetarians as compared to 103.2 μg/ml. in non-vegetarian, frequent meat eaters. The difference, however, was not significant. Nearly 65% of the mothers had milk vitamin B12 values below 100 μg/ml.

FAA content of milk measured as L. casei activity ranged from 1.0 mμg./ml. to 48.1 mμg./ml., with a mean value of 9.2 mμg./ml. With S.

### TABLE I

**Serum Vitamin B12 and Folic Acid Activity (FAA) in Indian Mothers**

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Hb (g./100 ml.)</th>
<th>Haematocrit (%)</th>
<th>Total Proteins (g./100 ml.)</th>
<th>Serum Vitamin B12 (μg/ml.)</th>
<th>Serum FAA (mμg/ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lacto-vegetarian</td>
<td>15</td>
<td>11.2 ± 0.5</td>
<td>34.0 ± 1.4</td>
<td>6.2 ± 0.1</td>
<td>97.3 ± 0.1</td>
<td>8.8 ± 1.2</td>
</tr>
<tr>
<td>Non-vegetarian, occasional</td>
<td>16</td>
<td>11.2 ± 0.4</td>
<td>33.8 ± 1.3</td>
<td>6.3 ± 0.2</td>
<td>181.6 ± 3.7</td>
<td>8.5 ± 1.3</td>
</tr>
<tr>
<td>Meat eaters</td>
<td>17</td>
<td>12.2 ± 0.4</td>
<td>36.8 ± 1.3</td>
<td>6.4 ± 0.1</td>
<td>228.3 ± 0.1</td>
<td>7.7 ± 0.1</td>
</tr>
</tbody>
</table>

Mean figures ± standard error.
faecalis R the values were significantly lower than those obtained with L. casei, where ascorbic acid was used in the extraction procedure. The average values for FAA did not differ significantly in the lacto-vegetarian and non-vegetarian groups. The majority (63.5%) of the mothers had FAA values below 10 mug./ml.

The average milk protein content was $1.58 \pm 0.06$ (SE) g./100 ml. There was no significant difference between the three dietetic groups.

**Discussion**

Previous studies in Indian mothers have shown that nutritional status can affect the birthweights of their infants; and the incidence of prematurity was high among the infants born to mothers of low socio-economic status (Kulkarni et al., 1959). In countries like India, where prolonged breast-feeding is practised, it is necessary to know the adequacy of breast milk as a sole nutritional item. The subjects studied belonged to a low socio-economic group. Previous studies on serum protein pattern in such mothers have revealed significantly low serum proteins and albumin, as compared to mothers belonging to high socio-economic status from the same population (Kulkarni et al., 1959). However, there is no striking difference in milk protein values between the poor Indian mothers and those reported from the other parts of the world (Macy, 1949; Macy and Kelly, 1964; Kon and Mawson, 1950; Gunther and Stanier, 1951; Escudero and Pierangei, 1952; Peters, 1952; Walker, Arvidsson, and Draper, 1954; Mayer, 1963). Belavady and Gopalan (1959) in a study of 191 mothers found no significant difference in milk composition, with regard to lactose and protein content, as compared to values observed among American, Australian, and British mothers, representing populations with a satisfactory state of nutrition. Similar results have also been obtained among Bantu, Chimbu, and New Hebridean women who probably represent a population with a less satisfactory nutritional status (cf. Belavady and Gopalan, 1959). The nutritional status of the mother, therefore, probably does not affect the chemical composition of milk as far as lactose and proteins are concerned. This is, however, not true with regard to vitamins. Thus, it was shown that as compared with British or American mothers, the vitamin A, thiamine, riboflavin, and vitamin C content of milk from poor Indian mothers was low (Macy, 1949; Macy, Kelly, and Sloan, 1950; Kon and Mawson, 1950; Gunther and Stanier, 1951). Our studies indicate that the vitamin B12 content of milk of Indian mothers is also very low as compared to values reported by.

**TABLE II**

*Serum Vitamin B12 and Haemoglobin Levels in Different Dietary Groups; Comparison Between Normal and Lactating Mothers*

<table>
<thead>
<tr>
<th>Group</th>
<th>Normal non-pregnant</th>
<th>Lactating</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Hb (g./100 ml.)</td>
</tr>
<tr>
<td>Lacto-vegetarian</td>
<td>38</td>
<td>12.6±</td>
</tr>
<tr>
<td>Non-vegetarian, occasional meat eaters</td>
<td>15</td>
<td>12.8±</td>
</tr>
<tr>
<td>Non-vegetarian, frequent meat eaters</td>
<td>32</td>
<td>12.6±</td>
</tr>
<tr>
<td>For all groups</td>
<td>12</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Mean figures ± standard error.
Western workers (Collins et al., 1951; Gregory and Holdsworth, 1955; Karlin, 1956; Davidov and Kruglova, 1960) (Table IV). Even though vegetarian subjects had low serum vitamin B12 levels, their milk vitamin B12 values were not significantly low as compared to those of non-vegetarian mothers. In fact no correlation existed between the vitamin B12 contents of serum and milk. It is possible, however, that the low maternal serum levels may lead to deficient laying down of B12 stores in the fetus.

Our results on milk FAA values are not comparable with the values reported by various workers perhaps because of the difference in the techniques used. The values quoted in the literature are usually obtained with S. faecalis R and hence are lower than our results. It is probable that the folate present in the milk is in reduced form, and that most of it is in a form utilized by L. casei (Ramasastri, 1965). It is known that pteroyl-triglutamate and methyl-tetrahydrofolate are utilized as growth factors only by L. casei and not by S. faecalis R (Usdin, 1959). The method using S. faecalis R as test organism is known to have low sensitivity and it probably does not measure all the folate activity present in the samples (Toennies, Frank, and Gallant, 1953; Herbert, 1961; Waters and Mollin, 1961; G Grossowicz et al., 1962). The results obtained in the present study with the use of improved media clearly show that the amount of S. faecalis R, active folate material present in human milk is small and that assays with L. casei would give a better picture of total folate activity. The average total folate values of 9.2 μg./ml. in transitional milk obtained in our study are substantially higher than the corresponding values obtained by earlier workers (Macy, 1949; Collins et al., 1951). The present values are similar to those reported by Ramsastri (1965) from India who obtained an average value of 8.4 μg./ml., using L. casei and ascorbic acid containing medium for analysis. The S. faecalis R activity constituted about one-third of the total activity estimated by L. casei. There was no correlation between the serum FAA concentration and the corresponding milk secretion.

Since milk is a poor source of iron, the commonest type of anaemia observed in Indian infants is due to iron deficiency. Among the megaloblastic anaemias, anaemia due to folic acid deficiency is probably more common (Chatterjea, 1967). However, it is possible that the deficiency of vitamin B12 may be a significant contributing factor. Wokes (1957) found that infants of ‘vegans’ did not show

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**Table III**

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Protein (g/100 ml.)</th>
<th>Vitamin B12 (μg/ml.)</th>
<th>FAA (μg/ml.)</th>
<th>FAA (μg/ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lacto-vegetarian</td>
<td>15</td>
<td>1.52 ±</td>
<td>91.3 ±</td>
<td>3.0 ±</td>
<td>4.1 ±</td>
</tr>
<tr>
<td>Non-vegetarian, occasional meat eaters</td>
<td>16</td>
<td>1.62 ±</td>
<td>100.4 ±</td>
<td>6.4 ±</td>
<td>2.0 ±</td>
</tr>
<tr>
<td>Non-vegetarian, frequent meat eaters</td>
<td>17</td>
<td>1.62 ±</td>
<td>103.2 ±</td>
<td>10.8 ±</td>
<td>3.3 ±</td>
</tr>
</tbody>
</table>

Mean figures ± standard error.

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**Table IV**

<table>
<thead>
<tr>
<th>Authors</th>
<th>Country</th>
<th>B12 (μg./ml.)</th>
<th>Range (μg./ml.)</th>
<th>Test Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collins et al. (1951)</td>
<td>U.S.A.</td>
<td>410</td>
<td>—</td>
<td>Lactobacillus leichmannii</td>
</tr>
<tr>
<td>Srinivas Murthy, Nambudripad, and Iysa (1953)</td>
<td>India</td>
<td>80</td>
<td>55-160</td>
<td>L. lactis</td>
</tr>
<tr>
<td>Karlin (1956)</td>
<td>France</td>
<td>300</td>
<td>—</td>
<td>L. leichmanni</td>
</tr>
<tr>
<td>Gregory and Holdsworth (1955)</td>
<td>U.S.A.</td>
<td>300</td>
<td>—</td>
<td>L. leichmanni</td>
</tr>
<tr>
<td>Deodhar and Ramkrishnan (1959)</td>
<td>India</td>
<td>92</td>
<td>82-113</td>
<td>L. leichmanni</td>
</tr>
<tr>
<td>Davidov and Kruglova (1960)</td>
<td>Russia</td>
<td>400</td>
<td>200-600</td>
<td>—</td>
</tr>
<tr>
<td>Lancet (1965)</td>
<td>India</td>
<td>100</td>
<td>40-390</td>
<td>E. gracilis</td>
</tr>
<tr>
<td>Present study</td>
<td>India</td>
<td>100</td>
<td>40-390</td>
<td>E. gracilis</td>
</tr>
</tbody>
</table>
any evidence of vitamin B12 deficiency as long as they were on breast milk but these symptoms appeared after weaning on to low protein diets and were controlled by cow's milk. Recently, a syndrome characterized by anaemia, abnormal skin pigmentation, mental retardation, and tremors has been described among Indian infants of 4–11 months (Dikshit, 1957; Pohowalla et al., 1960; Kaul, Prasan, and Chowdhry, 1963; Jadhav et al., 1962; Srikantia and Reddy, 1967). The bone–marrow in such infants usually showed megaloblastic erythropoiesis, and the serum vitamin B12 levels were low. Administration of vitamin B12 in such cases, either by increasing the infant's intake of milk or by raising the concentration of vitamin B12 in the milk, could correct the blood picture and also the clinical manifestation to varying degrees (Srikantia and Reddy, 1967). The need for further studies for finding out the incidence of B12 deficiency in Indian infants is thus obvious.

REFERENCES


Maternal Milk and Serum Vitamin B12, Folic Acid, and Protein Levels


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