ELEVATED RED CELL VOLUME IN NORTH INDIAN STUDENTS

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Abstract. In a survey for beta-thalassemia carrier status among students in the State of Punjab in India, a surprisingly large number were found to have an elevated red cell volume over 99 fl. The finding was predominantly but not exclusively in females. Similar student surveys from other states showed less macrocytosis. Follow-up tests in a group of affected students were carried out. Volunteers were asked to modify their diet then after six months they were provided with oral vitamin B_{12}. The resulting changes are reported and the implications of the probable vitamin B_{12} and or folic acid deficiency are considered.

INTRODUCTION

Ludhiana is one of six centers in India for the Jai Vigyan Thalassemia Project, which is government sponsored and organized through the Indian Council of Medical Research (ICMR). The aims are to raise awareness of the disease and to reduce the number of births with thalassemia major. The initial phase (commencing in 2001) involved each center screening 5,000 students, age 18-23, for carrier status. The blood tests included an automated complete blood count. In addition 5,000 pregnant women were tested, and where thalassemia trait was demonstrated, blood examination of the partner was arranged. The students screened came from medical and dental schools, higher educational institutions and nursing colleges in Ludhiana with a population of 3 million. Where carrier status was shown appropriate counselling was carried out by trained social workers.

While screening, a surprising number of participants were found to have an elevated red cell volume. Fig 1 shows 1 in 5 subjects tested at a nursing college had an MCV of 100 fl or above. In none of those with an elevated red cell volume was bata-thalassemia trait demonstrated. ICMR surveys in other parts of India showed a lower proportion of students with an elevated MCV than in Punjab (unpublished data). The macrocytosis was found predominantly but not exclusively among female students. Where abnormalities were demonstrated the subject was informed and given advice in the management. It was felt that vitamin B_{12} and or folic acid deficiency were the cause of this finding, the most likely cause being a predominantly vegetarian diet. Students were educated on modifying their diets then vitamin B_{12} was given in those with MCV>99 fl.

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MATERIALS AND METHODS

The study was carried out at two nursing colleges with the co-operation of the senior staff. The unlikely possibility that some other cause for macrocytosis than vitamin deficiency was kept in mind. Meetings were arranged to which all students with an MCV of 100 fl or greater were invited. An explanation of our findings was given. At the first interview we recommended they increase their intake of green vegetables and fresh milk and volunteers were requested to give a further blood

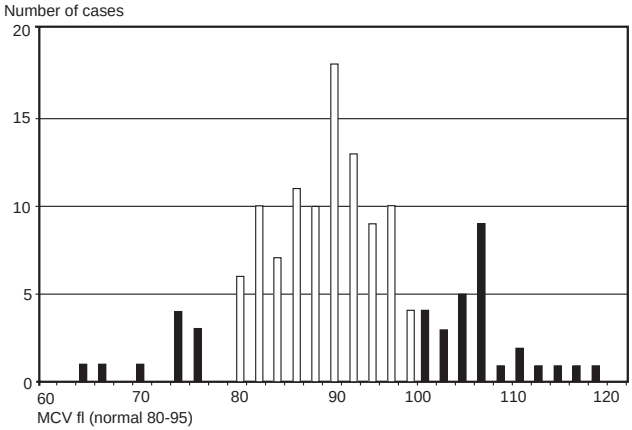

Fig 1–The distribution of mean red cell volumes in 138 nursing students at college 2.

sample after six months. Each volunteer was interviewed to determine type of diet, presence of health problems and place of origin, urban or rural.

Nine of the original 20 volunteers followed up and blood samples were taken for complete blood count and serum vitamin B_{12} and folate levels. Each student was given vitamin B_{12} tablets (Mecobal 500 µg) and instructed to take them once a week for 3 months. Six months later (3 months after completion of the oral vitamin) blood samples were obtained from 6 out of the 9 original participants (3 had completed their studies and moved away). In the 6 remaining subjects samples were taken after another 8 month interval. After completion of all the tests each student was sent a follow-up letter with their results with the suggestion they take a weekly vitamin supplement (we recommended Udervit ZC containing 15 µg vitamin B_{12} and 1.5 mg of folic acid). Discussions were held with the senior staff at each institution regarding modification of the canteen diet.

The complete blood counts were processed on the same day samples were obtained using the Abacus automated cell counter (Abacus Diatron, Ameisgasse, Austria). A quality control blood of known values was processed at the same time in each batch. A blood film was examined with May-Gr,nwald-Giemsa. Vitamin B_{12} and folic acid assays (Simul TRAC-SNB, ICN Diagnostics, Orangeburg, USA) were performed on serum samples. Samples showing hemolysis were rejected for the folate assay. Statistical significance regarding changes in the blood counts was calculated using paired *t*-test analysis of the data (Lewis, 1992).

RESULTS

Nine student nurses were included in the study with ages ranging from 18-23 years of whom seven were vegetarian and two non-vegetarian. Six originated from villages and 3 from urban areas. Up on initial interview none complained of symptoms or admitted any health problems. One of the six girls who completed the study stated she felt improvement by changing her diet and the course of tablets. None of the other students felt they had achieved a permanent change in eating habits.

The pre-treatment mean hemoglobin of the group was 11.5 g/dl, 5 of whom had a level less than 12.0 g/dl (serialized blood counts are shown in Figs 2 and 3). The initial mean MCV was 110 fl. Six months after beginning the dietary advice the mean hemoglobin was unchanged there was a significant fall in the mean MCV to 100 fl ($p = 0.001$). After vitamin B_{12} therapy a fall in MCV and a rise in hemoglobin were seen but did not reach statistical significance. The final blood count showed a reversal of these trends. Serum vitamin B_{12} and folate levels were assayed on one or two occasions for the subjects studied (Fig 4). The initial sample was taken six months after beginning dietary changes. The majority (7 out of 8) showed borderline or low vitamin B_{12} levels, which rose following oral vitamin B_{12} tablets. One student (No. 6) at the time of the

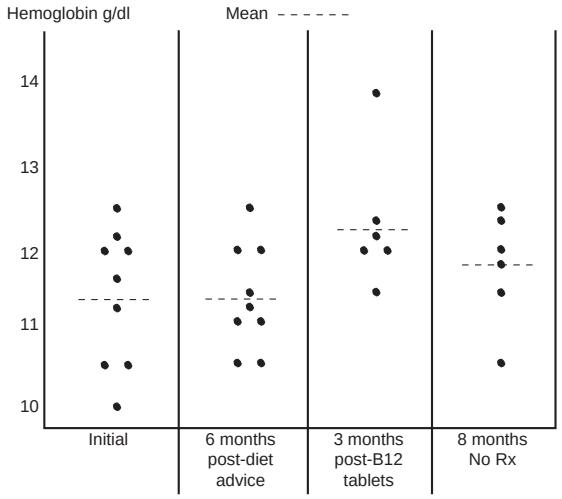

Fig 2–Hemoglobin levels during the 20 months of the study.

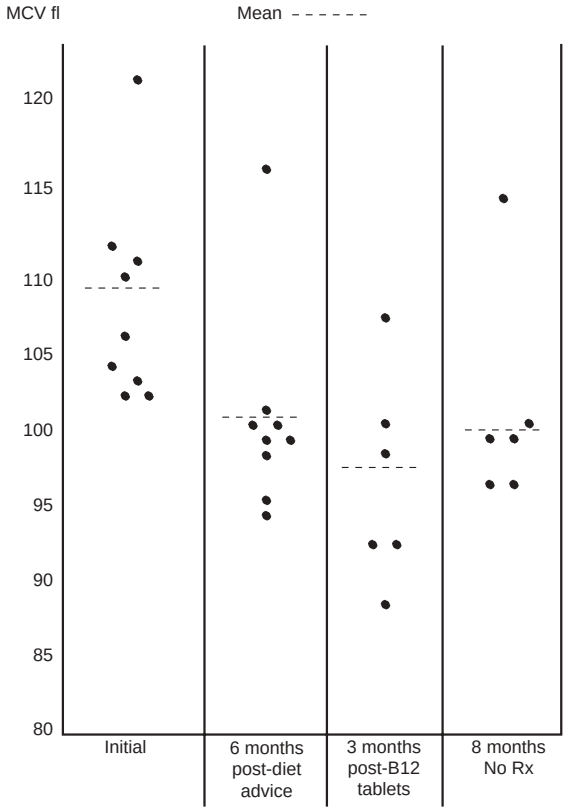

Fig 3–MCV levels during the 20 months of the study.

first vitamin assay blood sample was discovered to have been receiving Ayurvedic medicines and the levels were very high but had fallen nine months later when these were discontinued. Blood films were examined during the study and mild megaloblastic features were seen on the first blood tests. The initial blood film of student 2 is shown in Fig 5.

DISCUSSION

The Jai Vigyan Thalassemia Project examining large numbers of blood counts on students has shown that in addition to the expected iron deficiency, surprisingly large numbers of young persons had evidence of vitamin B_{12} and/or folic acid deficiency amounting to what may be a significant public health problem. It was encouraging that having counselled these young people to change their diet, increasing green vegetables and milk, that there was a clear-cut response shown by a fall in MCV. However, the enthusiasm waned over time. The decline in MCV did not persist and in only two subjects at eighteen months did the final MCV get near the upper limits of normal (95 fl), and only one of these subjects was confident her diet had improved. This emphasises how difficult it is to change dietary habits in the community and gives support to the need for possible fortification of foods with vitamins. The role of vitamins remains uncertain in this study. The blood sample taken after the trial of dietary change gave credence to the importance of increasing vitamin B_{12} intake (Fig 4). This was again emphasised by improving MCV levels following vitamin administration.

The importance of vitamin B_{12} deficiency has been recognized in this part of northern. India. Detailed investigations of megaloblastic anemia found in migrants from Punjab to London were carried out (Stewart *et al*, 1970; Britt *et al*, 1971). Many young adults were found to have nutritional vitamin B_{12} deficiency.

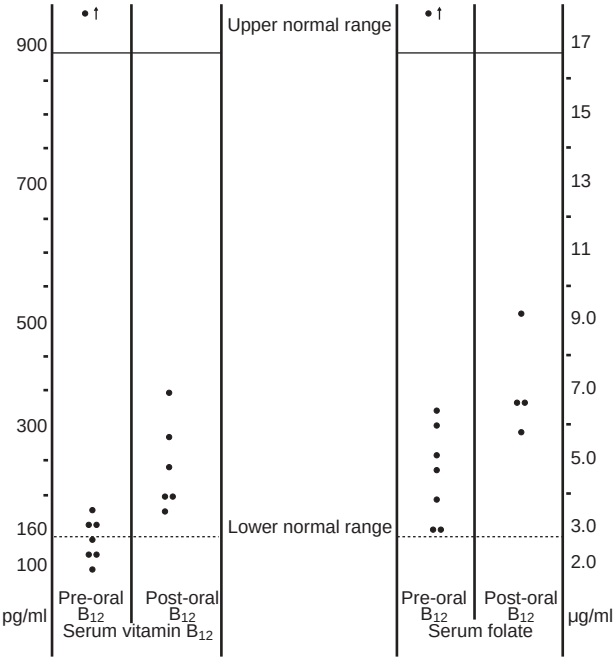

Fig 4–Serum vitamin B12 and folate levels estimated before and after oral vitamin B12 supplementation. One student with very high levels initially was taking Ayurvedic medicine.

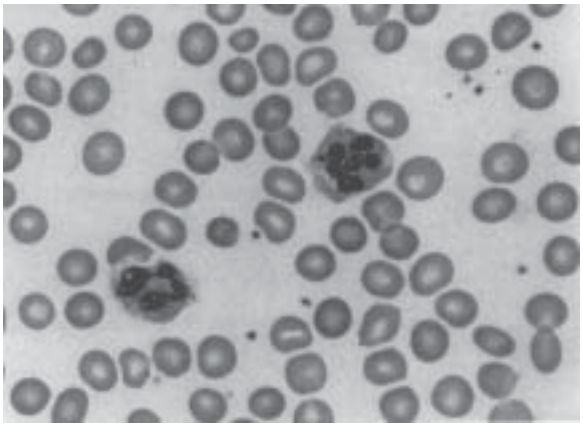

Fig 5–Initial blood film of one of the student nurses showing anisocytosis and macrocytosis of the red cells and a hyper-segmented neutrophils (x 1,000).

More recently, Sarode *et al* (1989) described nutritional vitamin B_{12} deficiency in northern India.

These young adults are at risk for the complications of vitamin B_{12} and folic acid deficiency. Neuropathy and central nervous system damage may result. Of special interest in this context in students is mental problems (Hoffbrand and Provan, 1997) with poor concentration, poor memory and depression which may reduce academic performance. A history and physical examination of these patients was not performed. A further project is planned in which a detailed neurological and psychiatric assessment will be included. There has been increasing interest in the elevated homocysteine levels found in both vitamin B_{12} and folic acid deficiency which may lead to vascular damage and be a risk factor for coronary heart disease (Urbink, 1994). In our study, students with macrocytosis were predominantly females; deficiency may have an influence on their reproductive health. Infertility is a recognized complication of deficiency of both these vitamins (Chanarin, 1990). Perhaps of even greater import is the clear link between maternal folic acid deficiency and neural tube defects in the fetus (Hibbard and Smithels, 1965; MRC, 1991).

There is recent evidence that fetal defects may result from maternal vitamin B_{12} deficiency (Ray and Blom, 2003). In Punjab, there was a USA-funded project to introduce folic acid supplementation for college female students (Asok, 2002, personal communication). In light of this evidence here and others that vitamin B_{12} deficiency is common we recommend that supplementation should be with a combination of vitamins. This would also eliminate the risk of folic acid treatment precipitating neurological damage in the presence of vitamin B_{12} deficiency.

The public health approach to nutritional deficiency in India has to be tackled on a nation-wide basis. The issue is complex and involves education to change dietary habits and improving the economic status of the population. In northern India there is no alternative

but supplementation for women with iron folic acid and vitamin B_{12} in reproductive years where abnormalities are demonstrated on blood count and blood film. This should commence before conception. Many women with bata-thalassemia trait we found to be iron deficient (unpublished data) so thalassemia would not be a contraindication to iron supplementation. Our findings may apply to some extent to other parts of India and to Asian migrants overseas.

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