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**THE PREVALENCE OF FOLATE AND VITAMIN B<sub>12</sub> DEFICIENCY  
IN AN ANAEMIC POPULATION**

**AND**

**FOLATE STUDIES IN LOCAL FOODS**

A THESIS PRESENTED

BY

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## ABSTRACT

This study focuses on the fact that even though megaloblastic (macrocytic) anaemia is common in Sri Lanka, it has not been studied whether it is mainly due to folate or vitamin B<sub>12</sub> deficiency (which are the main causes of it) and on the fact that information on Sri Lankan food folate and the effects of Sri Lankan culinary practices on the food folate content, is scarce. The patients are usually treated blindly with oral vitamins and vitamin B<sub>12</sub> injections.

The main objectives were to study

1. the folate status among a group of anaemic patients,
2. whether there is a significant relationship between poor culinary practices & food habits and folate deficiency,
3. the folate levels in some common Sri Lankan foods
4. culinary practices that give maximal dietary folate.

Serum and whole blood samples of two hundred anaemic and non-anaemic subjects were analyzed for folate. The anaemic patients were all new patients attending the Haematology Clinic, Teaching Hospital Kandy during a given period, who were not on any haematinic drugs. The cut off haemoglobin levels for males was 13 g/dl and for females it was 12 g/dl according to criteria given by the WHO. The age group selected was from 18 years – 60 years. Two hundred non-anaemic subjects matched for the age,

sex, socio economic status and ethnic group of the patients, as far as possible, were taken as the control group.

Personal data, medical history, drug history, family history, social history and dietary habits of each subject (patient/control) was obtained via a questionnaire.

Eighty seven Sri Lankan foods were studied. They included cereals, pulses, green leafy vegetables, non-leafy vegetables, roots, tubers, fruits, animal products, king coconut water, coconut water, tea and supplementary foods such as Triposha, Samaposha and Marmite. Nine samples of each food were analysed in triplicate.

Some of these foods were processed (heated, boiled, germinated, made into salads) and the effect of food processing was analyzed.

Folate levels were measured using a microbiological technique. The organism used in the assay was *Lactobacillus casei* (subsp. *rhamnosus*) ATCC 7469. The serum vitamin B<sub>12</sub> level was analyzed in the patients using an enzyme immunoassay.

The anaemic patients were categorized as mildly anaemic (haemoglobin 10.1-12.0g/dl), moderately anaemic (haemoglobin 7.1-10.0g/dl) and severely anaemic (haemoglobin <7.0g/dl) according to the World Health Organization, 1989. Most patients (43.5%) were moderately anaemic. There were more females than males among the mildly anaemic, moderately anaemic and severely anaemic groups.

There were more patients (56.5%) who had macrocytic red cells than those with microcytic, mixed or normocytic cells. The mean haemoglobin concentration of the patients was  $7.78 \pm 2.26$  g/dL and of the controls was  $13.2 \pm 1.06$  g/dL.

The cut off value (lowest level of the normal range) of 3.5 ng/ml for serum folate was calculated after considering a preliminary study of 50 normal subjects who attended the Lipid Clinic conducted at the Department of Biochemistry, Faculty of Medicine, University of Peradeniya and the serum folate values of the 200 control subjects. The cut off value for red cell folate was 160ng/ml which was the value given by DIFCO laboratories, the manufacturer of culture media used for the assay in this study. Subjects with a serum folate concentration  $< 3.5$  ng/ml or a red cell folate concentration below 160 ng/ml or both, were considered as being folate deficient.

One of the striking features among the patients in this study is the higher prevalence of folate deficiency (77%). Eighty per cent (159) of the patients had macrocytic anaemia and 154 (77%) were deficient in folate. The mean serum folate concentration in patients was  $3.42 \pm 2.14$  ng/ml.

Only one control subject was deficient in folate (Serum folate-normal; Red cell folate-low). The mean serum folate concentration in the controls was  $6.36 \pm 2.72$  ng/ml.

In spite of the abundance of vegetables and green leaves in Sri Lanka , poor dietary habits seem to contribute immensely, to the high prevalence of folate deficiency observed in this study. Around 86.4 % of the folate deficient patients adopted poor culinary practices and food habits.

There was a low prevalence of vitamin B<sub>12</sub> deficiency among the patients in this study. Only 12 (6 %) patients had low serum vitamin B<sub>12</sub> levels.

The study on foods was to find out the highest folate contributors among Sri Lankan foods and the effects of common food processing techniques on their folate concentration. The highest folate contributors were Yeast extract (Marmite), liver and sprouted beans, while egg yolk, green leaves and pulses such as red cowpea were also good sources. Mature green leaves had a lower folate concentration than young leaves.

Heat reduces the food folate significantly. (With a T test, the P value is less than 0.001 for each food article, which means that the difference is highly significant).

It was seen that the addition of lime and other ingredients to the leaves before heating, has a significant beneficial effect on the preservation of food folate, making the folate more stable. Germination of beans increased the folate content markedly ( $\approx$  x14). Young green leaves had a significantly higher concentration of folate than the mature leaves.