A Study of Serum Vitamin B12 and Folate Levels in Patients of Megaloblastic Anaemia in Northern Pakistan

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Introduction

Vitamin B12 and Folate play akey role inDNA synthesis in the dividing cells of the body. Deficiency of one or both of these results in reduced synthesis of DNA1,2. This leads to megaloblastic anaemia characterised by a characteristic pattern of morphological and functional abnormalities in peripheral blood and bone marrow cells[1,3,4s]. Interpretation of the serum levels of vitamin B,2 and Folate are difficult due to a wide range in their normal and cut off values in healthy individuals, Serum levels may also vaiy due to food habits and socio-economic conditions[5]. It ‘is, therefore, important to determine the serum levels of these vitamins in particular population before interpreting the results in patients of megaloblastic anaemia. A previous study demonstrated that the levels of both vitamins were lower in local population as compared to other populations[6]. It is therefore expected that levels of vitamin B12 and folic acid may also be different in patients with megaloblastic anaemia in our population.

Patients, Methods and Results

Fifty consecutive patients with megaloblastic anaemia diagnosed over a period of 21 months (Januaiy, 1988 to October, 1989) are included in this study. The diagnosis of megaloblastic anaemia was made by examination of peripheral blood andbone marrow aspirates. Major cnteria used for diagnosis were presence of anaemia, a high MCV, presence of macro-ovalocytes and hypersegmented neutrophils in penpheral blood film, megaloblastic eiythropoiesis and dysmyelopoiesis in the bone marrow and an elevated lactate dehydrogenase levels in serum in the absence of haemolysis. Four ml blood was collected from each patient into a sterile plain container. Serum was separated within two hours and frozen at -70°C in a cryotube until assays were done, Serum vitamin B12 and folate were estimated by radioimmunoassay technique. Amersham’s vitamin B, 2Ifo-late dual RJAkit(codes cT 301/CT 302) was used (Amersham International, U.K.). Of 50 patients 33, were males and 17 females. Theirages ranged from 6 months to 70 years (Mean 24 years). Serum vitamin B12 levels were 10-240 pg/nil (Mean±SEM values 96.3±8.94 pg/mi). One patient had a very high serum vitamin B,2 level (800 pg/mi) and was excluded from calculations. Serum folate levels ranged from 0.5 to 5 ng/ml (Mean±SEM values 2.7±0.21 ng/ml). The results are shown in Table.

Table. Results of Vitamin B_{12} and folate estimation in patients

Level	Number of patients	Percent
Serum B_{12} alone		
<145 pg/ml	28	56
145 pg/ml	1	2
Serum Folate alone		
<1.8 ng/ml	4	8
1.8 ng/ml	3	6
Combined deficiency	10	20
Total	46	92

Comments

Lower limit of serum vitamin B12 in healthy individuals varies from 150 to 300 pg/ml and the upper limit from 635 to 925 pg/nil[7,8]. In our study of normal individuals[6], lowest level was 110 pg/mI while the highest was 320 pg/nil. Both the levels are significantly lower than those described in literature and the range is also narrow. If we calculate the reference range to include 95% normal population it comes to 145 to 320 pg/mi. If this range is accepted as normal for the population of the area of study then 36(72%) patients fall below the lower limit. Serum folate levels in healthy individuals showed a much wider scatter. The lowest value was 1.3 ng/ml and highest 7.0 ng/ml. The lower limits of normal are reported to vary from 1.7 to 6 ng/ml[9,10]. The upper limit of normal in this series was almost one third of that described in literature. If a cut off point is selected to include 95% of normal individuals then the lower limit for our normal population is 1.7 ng/ml, which is similar to previous studies. if this is the true picture in general population of the study area then only 12 (24%) patients of megaloblastic anaemia have serum folate below normal range. Out of these 12 patients, only 4 (8%) had pure folate deficiency while 8 (16%) had deficiency of both vitamins. The remaining 10 (20%) patients were neither vitamin B12 nor folate deficient even by criteria established from our own study. However, 3 patients have folate levels exactly corresponding to the exact lower limit of the normal and one who had serum vitamin B12 level at the exact lower limit of normal. If these are included in respective groups then only 6 (12%) patients remained with levels within the reference ranges. This study shows that majority of patients of megaloblastic anaemia in Pakistani population have deficiency of vitamin B12. This is contrary to general belief that folate deficiency due to prolonged cooking and boiling is a more common cause of megaloblastic anaemia in Pakistan. Majority of our population is of low income group and does not have easy access to animal proteins which are the only source of vitamin B12. This fact is likely to lead to low body stores of vitamin B12 which is reflected in the relatively lower levels in blood. Development of malabsorption in such individuals would lead to much quicker lowering of the levels of these vitamins causing megaloblastic anaemia. Presence of normal levels of serum vituamin B12 and folic acid despite megaloblastic marrow cannot be explained by the present study. It is possible that these patients suffered from haemolytic anaemia with increased utilisation of one or

both of these vitamins.

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