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VITAMIN B₁₂ DEFICIENCY—A MAJOR CAUSE OF MEGALOBlastic ANAEMIA IN PATIENTS ATTENDING A TERTIARY CARE HOSPITAL

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Background: Folate and vitamin B₁₂ deficiencies have been known to cause megaloblastic anaemia. Since the deficiencies of these two vitamins are very common in Pakistani population, it would be imperative to investigate their role in causing megaloblastic anaemia. The objective of this study was to find out the contribution of folate and vitamin B₁₂ deficiencies in causing megaloblastic anaemia in our patient population. **Methods:** In this retrospective cohort study, clinical records of 220 patients (101 females and 119 males with an age range of 1–80 years) who presented themselves with macrocytic anaemia at the Aga Khan University Hospital were collected. Data pertaining to complete blood count and serum levels of folate and vitamin B₁₂ were analysed. **Results:** The mean haemoglobin (Hb) level was 6.8±0.2 gm/dl. Sixty-nine percent of the patients had severe anaemia (Hb<8 gm/dl). Mean±SEM values of haemoglobin, serum folate and serum B₁₂ were not significantly different between males and females (Hb 6.4±0.3 gm/dl vs 6.3±0.3 gm/dl; folate 6.9±0.8 ng/ml vs 7.8±1 ng/ml; B₁₂ 259±65 µg/ml vs 225±45 µg/ml, respectively). Linear regression analysis showed that serum folate was inversely related with the mean corpuscular volume (MCV, $p=0.04$). Spearman's correlation analysis indicated an inverse mild association between MCV and serum folate (correlation coefficient= -0.18). Folate deficiency was 43.4%, while vitamin B₁₂ deficiency was 78.5% in these patients. Seventy-one percent of folate-deficient patients had vitamin B₁₂ deficiency as well, while 26.1% of patients with B₁₂ deficiency had a co-occurrence of folate deficiency. **Conclusion:** Vitamin B₁₂ deficiency appears to be the major factor leading to megaloblastic anaemia in our study population. Inadequate dietary intake, over-cooking of our food and poor absorption might be contributing to high prevalence of vitamin B₁₂ deficiency in this population.

Keywords: Cobalamin deficiency, folate deficiency, macrocytic anaemia, megaloblastic anaemia, nutritional anaemia, vitamin B₁₂ deficiency, Pakistan

INTRODUCTION

Deficiencies of folic acid and vitamin B₁₂ are known to cause megaloblastic anaemia, an anaemia which is characterised by presence of abnormally large erythrocyte precursor cells, megaloblasts, in the bone marrow and macrocytic red cells in the peripheral blood.¹ These megaloblasts arise because of impaired DNA synthesis followed by ineffective erythropoiesis.¹

Megaloblastic anaemia is frequently seen in clinical practice in Pakistan.^{2,3} However, there have been only a few comprehensive reports regarding its prevalence, causative factors and associated symptoms in Indo-Pak subcontinent.²⁻⁶ We had reported previously that vitamin B₁₂ deficiency was the major cause of megaloblastic anaemia in patients who had been treated at the Aga Khan University Hospital for anaemias of vitamin deficiencies.² Since then, there have been hardly any detailed studies in this region on role of folate and vitamin B₁₂ in causing megaloblastic anaemia. The objective of the present study was to find out the contribution of folate and B₁₂ deficiencies in causing megaloblastic anaemia in our patient population.

PATIENT AND METHODS

It is a retrospective cohort study. Demographic and clinical information of 220 patients (age 1–80 years) with macrocytic anaemia who had been treated with B

vitamins for anaemias at the Haematology Clinic of the Aga Khan University Hospital (AKUH) during the past 15 years (Jan 1989 to Jun 2004) was obtained from the Department of Health Information Management Services, AKUH. The diagnosis of macrocytic anaemia was based on mean corpuscular volume >96 fl and history of the physical examination. Blood counts were performed with a Coulter Counter. Anaemic patients with the primary disease, such as hepatic disease, haemolytic anaemia, cancer, aplastic anaemia, myeloproliferative disease, red cell aplasia, multiple myeloma, leukaemia, chronic lung disease, chronic kidney disease and those using immunosuppressive or chemotherapeutic drugs were excluded from the study. Pregnant females, alcoholics and children below the age of 1 year were also excluded. Serum levels of folic acid and vitamin B₁₂ had been carried out by the Dual Count Kit (Diagnostic Products Corporation, Los Angeles, CA, USA) using proper controls. Vitamin B₁₂ deficiency, was defined as serum levels of B₁₂ ≤200 µg/ml. Folate deficiency was defined as serum levels of folic acid ≤3.5 ng/ml. Samples having border-line values of these vitamins were repeated. The study had been approved by the Ethics Review Committee of the Institution.

All analyses were performed on SPSS. Values of quantitative variables were presented as

Means±SEM, while proportions were given for categorical characteristics. Chi-square analysis was used to compare percent patients with vitamin deficiency with percent patients with vitamin-normal status. Linear regression analysis was performed to determine the nature of relationship between levels of vitamin B₁₂, folate and haemoglobin with mean corpuscular volume (MCV), adjusted for age. Correlation between these quantitative variables was assessed using Spearman's rank correlation test and $p < 0.05$ was considered significant.

RESULTS

Demographic and clinical characteristics have been shown in Table-1. Nearly 94% (n=204) of the patients were non-vegetarians. Bone marrow examination revealed that most of the patients had significant megaloblastosis. Mean age of male patients was marginally significant compared to female patients. In terms of haemoglobin concentration, serum folate and vitamin B₁₂ levels, there were no significant differences between males and females. Percent folate and vitamin B₁₂ deficiencies were 43.4% and 78.5%, respectively (Table-2), while among the vegetarians, percent vitamin B₁₂ deficiency was nearly 85%. Seventy one percent of folate-deficient patients had vitamin B₁₂ deficiency as well, while 26.1% of vitamin B₁₂-deficient patients were having a co-occurrence of folate deficiency. Linear regression analysis revealed inverse relationship between serum folate and MCV ($p=0.04$). Strength of this relationship was determined using Spearman's correlation test which indicated an inverse mild association between serum folate and MCV correlation coefficient= -0.18; (Table-3).

Table-1: Demographic and clinical characteristics of patients Mean±SEM

Variable	Total	Male	Female	p-value*
Age (years)	38.9±1.8	42.6±3.4	36.7±3.6	0.05
Haemoglobin (g/dl)	6.8±0.2	6.4±0.3	6.3±0.3	0.81
MCV (fl)	103.5±1.1	106.2±1.8	104.1±2.1	0.13
Serum vitamin B ₁₂ (pg/ml)	246.6±33	225±45	259±65	0.48
Serum folate (ng/ml)	7.5±0.6	6.9±0.8	7.8±1.0	0.84

*p-value compares the Mean±SEM values of males and females by Independent samples t-test.

Table-2: Frequency distribution of patients with respect to their B vitamin status

Vitamin B status	Total n(%)	Male n(%)	Female n(%)	p-value**
Folate				
-Deficiency (≤3.5 ng/ml)	56*(43.4)	32(43.8)	24(42.8)	0.13
-Normal (>3.5 ng/ml)	73(56.6)	41(56.2)	32(57.2)	
Vitamin B₁₂				
-Deficiency (≤200 pg/ml)	153*(78.5)	86(79.6)	67(77.0)	<0.001
-Normal (>200 pg/ml)	42(21.5)	22(20.4)	20(23.0)	

*40 patients in each of these groups had deficiencies of both folate and vitamin B₁₂. **Test of proportions using chi square was used to compare folate-deficient patients with folate-normal patients and B₁₂-deficient patients with B₁₂-normal patients.

Table-3: Correlations of age, haemoglobin, vitamin B₁₂ and folate with MCV

Variable	Correlation Coefficient*	p-value
Age (years)	0.14	0.31
Haemoglobin (g/dl)	-0.13	0.16
Serum vitamin B ₁₂ (pg/ml)	-0.03	0.71
Serum folate (ng/ml)	-0.18	0.04

*Correlation analyses were carried out by Spearman's correlation test

DISCUSSION

Folic acid and vitamin B₁₂ are essential dietary components for humans, because they are required for DNA synthesis.⁷ Haematopoietic cells (having a very high turnover) are especially sensitive to deficiencies of folate and vitamin B₁₂. With derangement of DNA synthesis due to deficiencies of these vitamins, megaloblastic anaemia ensues. Since folate and vitamin B₁₂ deficiencies are quite common among Pakistani individuals⁸, these could be the major factors for megaloblastic anaemia in our population. In a recently reported small study (n=50) on a hospital-based population of patients with megaloblastic anaemia at the Pakistan Institute of Medical Sciences, Islamabad, the contribution of folate and B₁₂ deficiencies was found to be 76%.³ The results of the present study show that vitamin B₁₂ deficiency is the major cause of megaloblastic anaemia in patients presenting with macrocytic anaemia at the Haematology Clinic of AKUH over a period of 15 years. These results add credence to our previous preliminary findings that vitamin B₁₂ deficiency rather than folate-deficiency is the major cause of megaloblastosis in a population that is largely non-vegetarian. Recently, we have shown that vitamin B₁₂ deficiency in an urban population in Karachi is nearly 10%.⁹ This is surprisingly a high figure considering that most of the Pakistani population is non-vegetarian. Four patients in the present study had severe gastritis. They were checked for intrinsic factor antibodies which were found to be absent in all four of them. This shows that pernicious anaemia is not very common in Pakistani patients with megaloblastic anaemia. More in-depth studies are required for determining the causes of B₁₂ deficiency in Pakistani population. The extent of B₁₂ deficiency in our patient population is similar to the one found by Khunduri and Sharma who have reported vitamin B₁₂ deficiency to be 65% in a hospital-based population in Delhi and anorexia and gastritis to be among the predominant symptoms.⁵ Similarly, Sarode *et al* have shown prevalence of vitamin B₁₂ deficiency to be 76% in 102 cases of nutritional megaloblastic anaemia in a hospital in Chandigarh.⁶ Though our results pertaining to high prevalence of vitamin B₁₂ deficiency in megaloblastic anaemia represent a patient population largely belonging to Southern Pakistan, yet a similar trend has

been reported previously by Mannan *et al* from Northern Pakistan.⁴ They have also shown that vitamin B₁₂ deficiency to be the major cause of megaloblastic anaemia in patients visiting the Armed Forces Institute of Pathology, Rawalpindi. In a small study from Combined Military Hospital in Gilgit (Northern Areas of Pakistan), prevalence of vitamin B₁₂ deficiency has been shown to be 31.8% and chronic giardiasis and dietary insufficiency appear to be the major causes for increased incidence of megaloblastic anaemia in that region.¹⁰ We are mindful of the fact that inclusion of patients in this study was based on high MCV (>96 fl), however, concomitant deficiency of iron (which is very common in Pakistani population) would impair identification of several cases of vitamin B₁₂ or folate deficiency on the basis of macrocytosis only.¹¹ Therefore, we suggest that prevalence of megaloblastic anaemia due to vitamin B₁₂ and/or folate deficiency could be even higher in Pakistani population. Severity of anaemia among our patients merits some discussion. Applying WHO criteria for anaemia, 69.1% of our patients were found to be severely anaemic (Hb <8 gm/dl). This indicates that majority of them approached the hospital when the disease had worsened to a great extent, and they must have been harbouring these vitamin deficiencies for several months. Apparently healthy people can have low levels of vitamin B₁₂ deficiency. With B₁₂ deficiency close to 10% in an apparently healthy general population in Karachi, it is suggested that serum B₁₂ screening should be seriously considered for individuals showing even minor symptoms of its deficiency.

Studies have shown an association of neuropsychiatric disorders with vitamin B₁₂ deficiency, especially in the absence of anaemia or macrocytosis,^{12,13} therefore, an early screening for vitamin B₁₂ has a merit, keeping in view the very high costs of late treatment of irreversible neuropsychiatric disorders arising from vitamin B₁₂ deficiency.¹³

CONCLUSION & RECOMMENDATIONS

Vitamin B₁₂ deficiency is a major factor leading to megaloblastic anaemia in our study population.

Inadequate dietary intake, over-cooking of our food and, perhaps, poor absorption could be contributing to high prevalence of vitamin B₁₂ deficiency in this population. Contrary to the general belief, vitamin B₁₂ deficiency or insufficiency appears to be quite common among Pakistanis. Medical community should seriously consider the merit of early screening (for vitamin B₁₂) of patients with minor symptoms of anaemia.

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