Frequency of Vitamin B12 and Red Cell Folate Deficiency in Macrocytic Anaemia

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Abstract: *Background*: Macrocytosis refers to a condition in which red blood cells are larger than normal, with or without anemia. Macrocytic anemia occur due to variety of illnesses and requires further clinical and laboratory assessment. Both folate and vitamin B12 have been known to cause macrocytic anemia since the deficiencies of these two vitamins are very common in Pakistan, it would be imperative to investigate their role in causing megaloblastic anemia.

Objective: To determine the frequency of vitamin B12 and folate deficiencies in cases of macrocytic anemia.

Material and Methods: This is a descriptive cross-sectional study conducted in the department of medicine of Abbasi Shaheed hospital from January 2012 to June 2012. A total of 95 patients (65 males and 30 females) with an age of above 13 years, who admitted with macrocytic anemia (MCV>96) with hemoglobin < 12 mg/dl in females and < 13 mg/dl in males were enrolled and data pertaining to complete blood count, serum level of vitamin B12 and RBC folate were analyzed.

Result: A total of 95 patients (65 males and 30 females) with hemoglobin < 12 mg/dl in females and < 13 mg/dl in males were taken. Mostly were non vegetarian and majority of the patients belongs to age group of 34-54 years. It was found that 69 patients (48 males and 21 females) from total of 95 had vitamin B12 deficiency which is 72.6% and 43 patients (20 males and 23 females) from total of 95 had folate deficiency which is 45.26%.

Conclusion: Our study concludes that vitamin B12 is a major contributing factor of macrocytic anemia in our population. Dietary insufficiency, poor absorption and increase demand might be contributing to high prevalence of vitamin B12 deficiency in our population. Therefore our medical community should seriously consider the merit of early screening for vitamin B12 deficiency and take precautions against the clinical consequences of vitamin B12 deficiency.

Keywords: Macrocytic anemia, vitamin B12, folate, megaloblastic anemia.

INTRODUCTION

The term Macrocytosis refers to a condition in which Red Blood Cells are larger than normal. It is a relatively common finding with prevalence ranging from 1.7% to 3.6% [1].

The most common cause of macrocytic anemia is Megaloblastic anemia which is the result of impaired DNA synthesis while RNA synthesis is unaffected. Vitamin B12 and folate coenzymes are required for thymidylate and purine synthesis, thus there deficiency can result in retarded DNA synthesis. Synthesis of DNA may also be delayed when certain drugs or chemotherapeutic agents are used for example folate antagonists (methotrexate), purine antagonist (6-mercaptopurine) and pyramidine antagonist (cytosine arabinoside) [2].

Non megaloblastic macrocytic anemias are those in which no impairment of DNA synthesis occurs. This category includes, accelerated erythroposis, alcoholism, hepatic diseases and hypothyroidism. Patients with hepatic disease and obstructive jaundice have macrocytosis secondary to increase cholesterol or phospholipids deposition on the membrane of circulating RBCS. In patients with hemolytic anemia or post hemorrhagic anemia the reticulocyte count increases, because of increase bone marrow activity. The reticulocyte, are immature RBC approximately 20% larger than more mature RBC [2], giving a macrocytic picture on a peripheral blood smear.

Vitamin B12 deficiency usually presents with various hematological, gastrointestinal and neuropsychiatric manifestations. Commonly seen neuropsychiatric manifestations include myelopathy, neuropathy, dementia, neuropsychiatric abnormalities and rarely optic nerve atrophy. Subacute combined degeneration (SCD) of the spinal cord is an uncommon cause of myelopathy but is the most frequent clinical manifestation of vitamin B_{12} deficiency. As anemia is a common early symptom leading to the diagnosis of vitamin B_{12} deficiency, neurological symptoms have often been considered to be late manifestations and typically occur after the development of anemia [3].

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Folate and vitamin B12 deficiencies have been known to cause megaloblastic anemia since the deficiencies of these two vitamins are very common in Pakistani population it would be imperative to investigate their frequency in causing megaloblastic anemia [4].

Present study is designed to find out the frequency of vitamin B12 and folate deficiencies in people of different ethnicity who are visiting a tertiary care hospital of Karachi because previously only few comprehensive reports are available regarding its prevalence in Indo-Pak subcontinent. Therefore, an early screening for vitamin B12 has a merit keeping in view the very high cost of late treatment of irreversible neuropsychiatric disorder arising from vitamin B12.

MATERIAL AND METHOD

This study was conducted in the department of medicine Abbasi Shaheed hospital from January 2012 to June 2012. It is a descriptive cross-sectional study with non-probability purposive sampling. All patients after giving informed consent were registered on a questionnaire. All patients were seen and examined by a physician. All patients underwent a detailed history and a complete physical and systemic examination.

Inclusion Criteria

All patients of > 13 years of age and either sex were included in the study. Patients with a hemoglobin of < 12 mg/dl in females and < 13 mg/dl in males with a mean corpuscler volume > 96 fl were included.

Exclusion Criteria

Pregnant patients, critically ill patients like sepsis, adult respiratory distress syndrome, patients on mechanical ventilation, patients with co morbidities like diabetes mellitus, hypertension, and ischemic heart disease were excluded. Patients on multiple drugs like folate antagonists, purine antagonists and pyramidine antagonists were also excluded.

All patients underwent a complete blood count with indices, serum vitamin B12 levels, red cell folate levels, chest X-ray, urine detailed report to exclude infection. A fasting and a random blood sugar to exclude diabetes mellitus and electrocardiogram to exclude ischaemic heart disease.

Diagnosis of Anaemia

In order to make a generalized approach to the diagnosis of anemia, the World Health Organization (WHO) has established a reference range for normal blood hemoglobin concentration, depending on age and sex. According to this criterion, anemia is present if the blood concentration of hemoglobin falls below 13 g/dl in men or 12 g/dl in women. This rule does not apply to infants, children and pregnant women, who have their own tables of lower limits of hemoglobin concentration. The WHO criterion has been accepted widely for diagnosis and publication, but its universal application has been questioned mainly because of racial differences [5, 6].

Diagnosis of B12 Deficiency

Serum cobalamin levels <150 pmol/l and clinical features and/or hematological anomalies related to cobalamin deficiency. OR serum cobalamin levels <150 pmol/l (<200 pg/ml) on two separate occasions [7, 8].

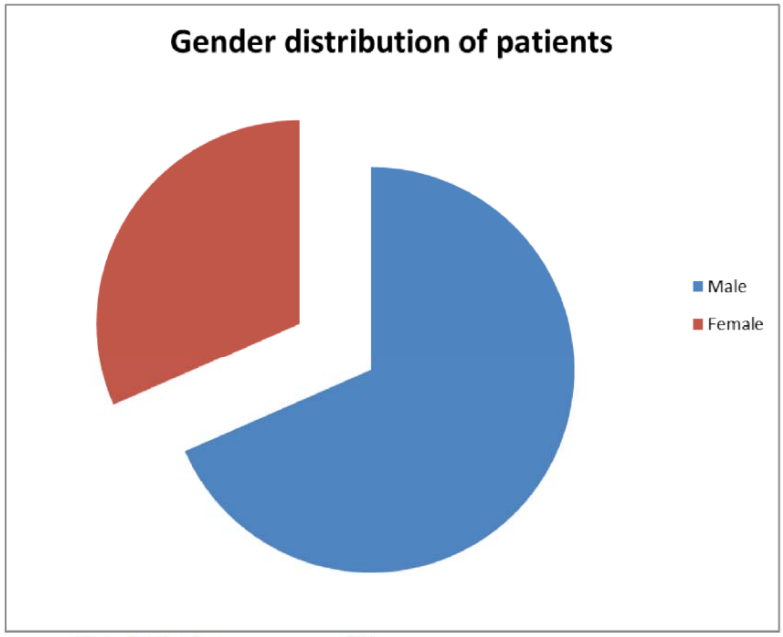
Data Analysis

All collected data have been entered into Statistical Package for Social Sciences (SPSS) version 11.0. Descriptive statistical analyses of continuous and categorical variables were performed. Data on continuous variables include age, laboratory investigation i.e. CBC, Serum B12, RBC folate, etc. were presented as Mean ± SD and data on categorical variables include gender, presenting complaints, use of alcohol, B12 deficiency and folate deficiency were presented as frequency and percentage.

RESULTS

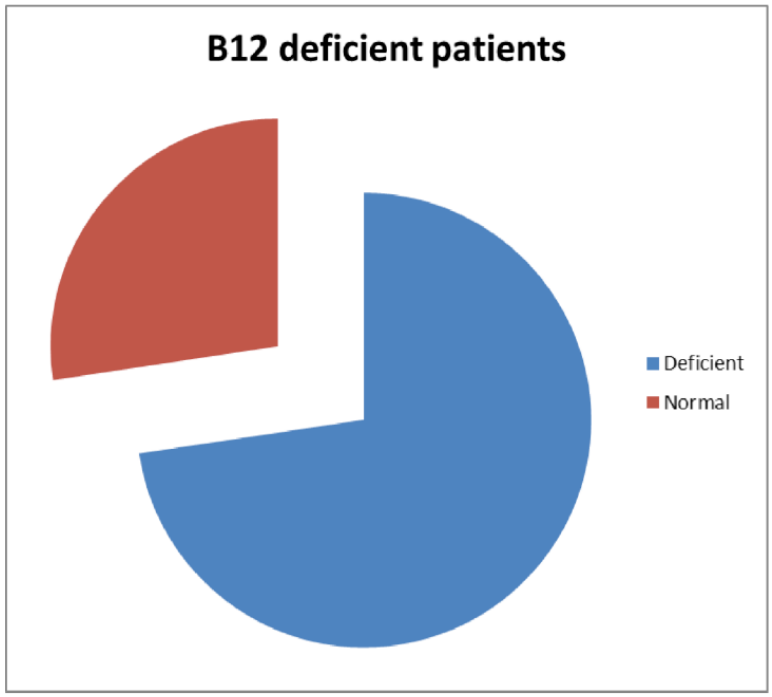
Patients with anemia admitted in Medical department of Abbasi Shaheed Hospital, Karachi was subjected to determination of complete blood count. Out of all, 95 patients with macrocytosis (MCV > 96fl) hemoglobin of < 12 g/dl in females and < 13 g/dl in males were included in the study. Among 95 patients, sex distribution showed 65 (68.4%) were males and 30 (31.5%) were females. (Figure **1**). Male to female ratio was approximately 2:1.

Majority of patients belongs to age group of 34-54 years (Figures **4** and **7**, Tables **1** and **2**). Mean age of the patients with B12 deficiency was found 52.33 ± 18.28 while in folate deficiency was 49.39 ± 19.67. Mean age of male patients with folate deficiency was marginally significant compared to female patients. In terms of hemoglobin, MCV, serum B12 and red cell folate levels, there were no significant differences between males and females.



Male Patients	65
Female Patients	30
Total Patients	95

Figure 1: Gender distribution of patients.



Deficient Patients	69
Normal Patients	26
Total Patients	95

Figure 2: B12 deficient patients.

It was found that 69 patients (48 males and 21 females) from total of 95 had vitamin B12 deficiency which was 72.6% while 43 patients (20 males and 23 females) from total of 95 had folate deficiency which was 45.26% (Table **3**).

Nearly 82% of patients were non vegetarian and among vegetarians 88% were B12 deficient (Table **4**). In this study only 2 patients were alcoholic and both were B12 and folate deficient.

Table 1: Age and Gender Distribution of Patients with B12 Deficiency

Age (Years)	Number of Patients			Percentage		
	Male	Female	Total	Male	Female	Total
13-33	9	3	12	13.04	4.34	17.38
34-54	15	11	26	21.74	15.94	37.68
55-75	18	4	22	26.08	5.79	31.87
Above 75	6	3	9	8.69	4.34	13.03

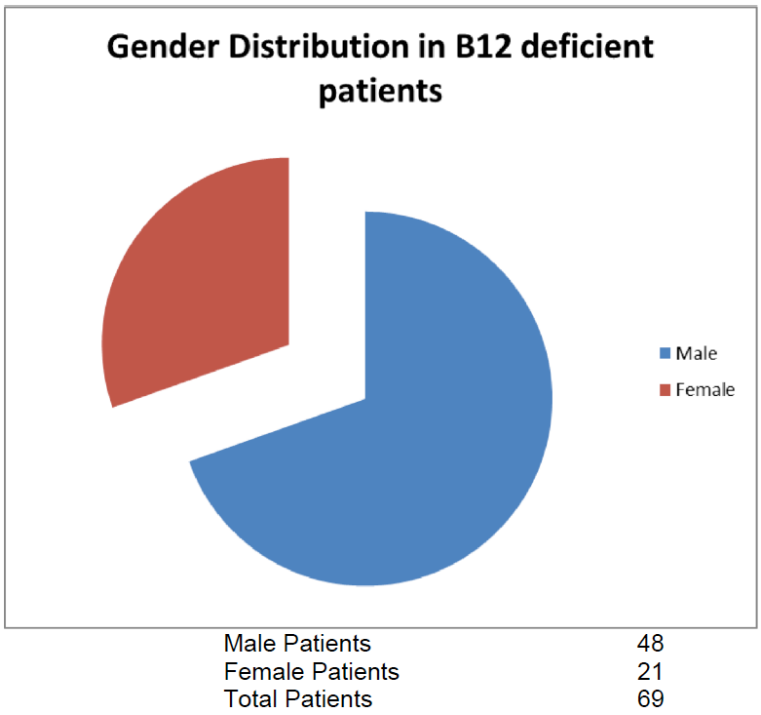


Figure 3: Gender distribution in B12 deficient patients.

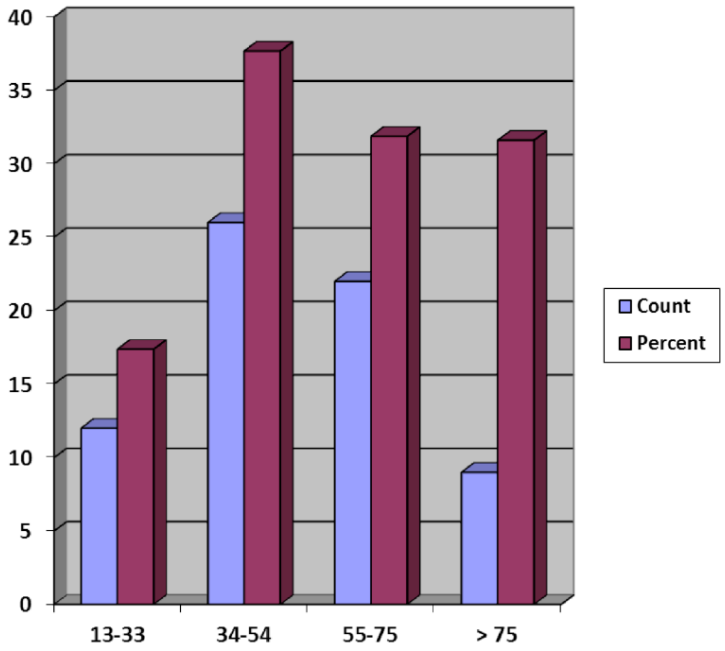
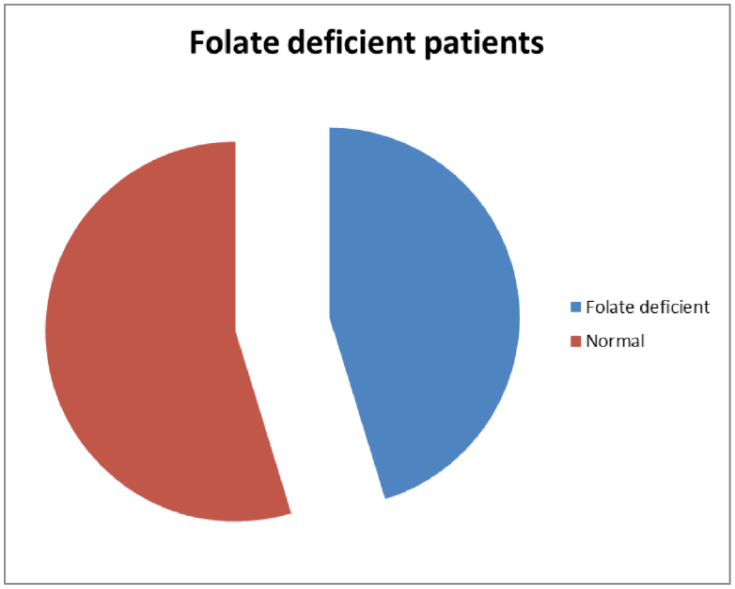


Figure 4: Age distribution of patients with B12 deficiency.

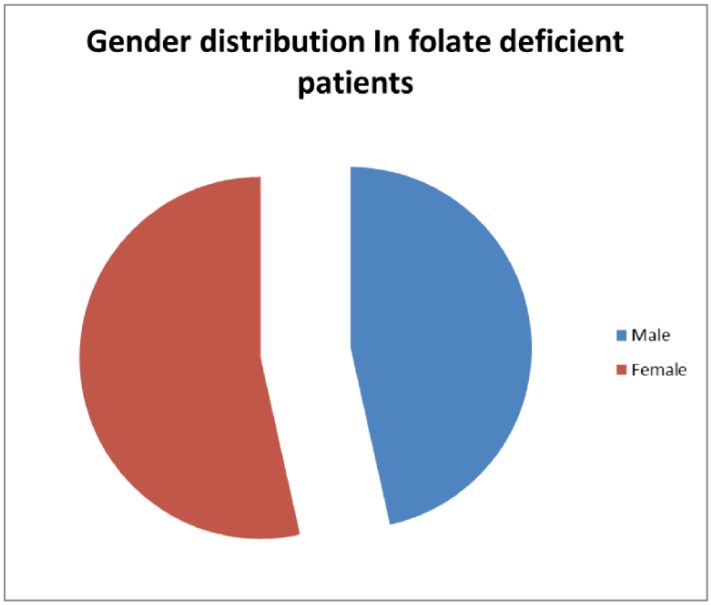


Folate deficient patients	43
Normal patients	52
Total Patients	95

Figure 5: Folate deficient patients.

Table 2: Age and Gender Distribution of Patients with Folate Deficiency

Age (Years)	Number of Patients			Percentage		
	Male	Female	Total	Male	Female	Total
13-33	2	7	9	4.65	16.27	20.93
34-54	8	10	18	18.60	23.25	41.85
55-75	6	4	10	13.95	9.30	23.25
Above 75	4	2	6	9.30	4.65	13.95



Male patients	20
Female Patients	23
Total Patients	43

Figure 6: Gender distribution in folate deficient patients.

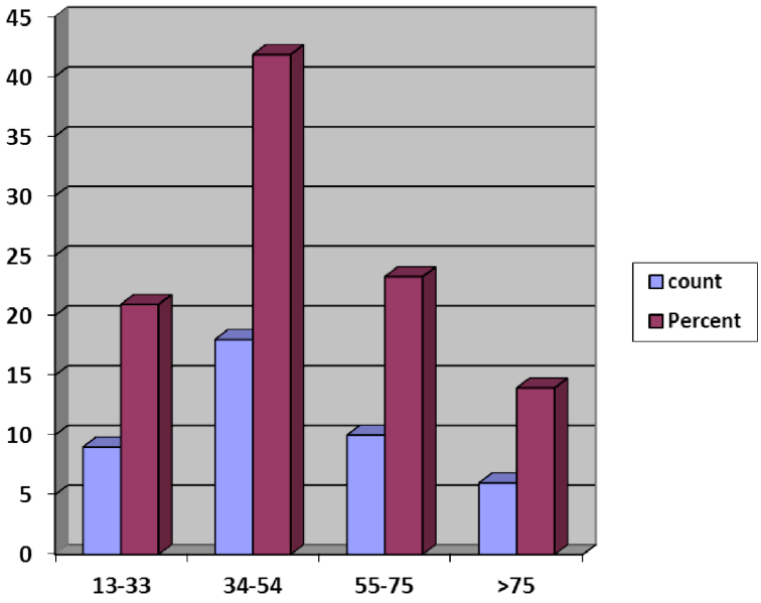
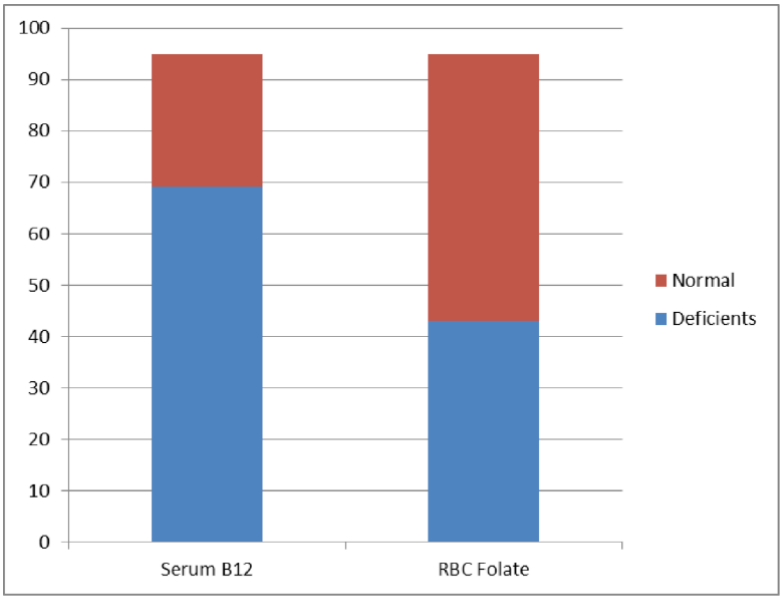


Figure 7: Age distribution of patients with Folate deficiency.

Table 3: Frequency Distribution of Patients with Respect to their Vitamin Status

Vitamin Status	Total		Male		Female	
	Count	Percent	CountPee	Percent	Count	Percent
Folate						
Deficient<150 ng/ml	43	45.26	20	46.5	23	53.58
Normal>150 ng/ml	52	54.7	45	86.5	07	13.46
Vitamin B12						
Deficient<170 pg/ml	69	72.63	48	69.56	21	30.43
Normal>170 pg/ml	26	27.36	17	65.38	09	34.61



	Serum B12	RBCFolate
Normal	26 (27.36%)	52(54.7%)
Deficient	69 (72.63%)	43 (45.26%)

Figure 8: Frequency distribution of patients with respect to their vitamin status.

Table 4: Serum B12 Deficiency Among Vegetarian and Non Vegetarian

	Vegetarian		Non Vegetarian	
	Count	Percent	Count	Percent
Total patients	17	18	78	82
B12 deficient	15	88.23	54	69.23

Total patients = 95 (vegetarian = 17, Non vegetarian =78).
Total B12 deficient = 69 (vegetarian = 15, Non vegetarian =54).

DISCUSSION

Macrocytosis due to vitamin B12 or folate deficiency is a direct result of ineffective or dysplastic erythropoisis. These important vitamins and cofactors are required for DNA synthesis. When either of these two factors is deficient, megaloblastic anemia can occur. Folate and vitamin B12 deficiency are quite common among Pakistani population. In one study done at Ayub Medical College the rate of non vegetarian group folate and B12 deficiency were 43.4% and 78.5% respectively while among the vegetarian B12 deficiency was nearly 85% [4]. Hashim *et al.* have shown prevalence of folate and B12 deficiency was 76% at Pakistan Institute of Medical Sciences, Islamabad [9]. Recently Yakub *et al.* have shown that vitamin B12 deficiency in an urban population in Karachi is nearly 10% [10]. This is surprisingly a high figure based on the fact that most of the Pakistani population is non vegetarian, our study results shows that vitamin B12 deficiency is the major cause of megaloblastic anemia. These results are similar to that which were found at Delhi by Khunduri that vitamin B12 deficiency was 65% in a hospital based population [11]. Our results largely represent urban population of southern Pakistan but a study done by Naeem *et al.* at Combined Military Hospital, Gilgit shows prevalence of vitamin B12 deficiency was 31.8% in Northern areas of Pakistan [12]. In another study published in World Applied Sciences journal in 2011 shows that vitamin B12 deficiency was 48% [13]. Limitations of this study include inability to document actual prevalence because of concomitant deficiency of iron would impair identification of several cases of vitamin B12 and folate deficiency as we include patients on the basis of high MCV(> 96fl).Therefore, we come to the conclusion that macrocytic anemia due to vitamin B12 and folate deficiency could be even higher than our results in Pakistani population.Early screening of vitamin B12 is important because of the fact that late identification will lead to irreversible neuropsychiatric disorder leading to increase morbidity of patients which ultimately causes increase health burden to the family and society. So, it is advisable to screen individuals showing minor symptoms of B12 deficiency.

CONCLUSION

Our study concludes that vitamin B12 is a major contributing factor of macrocytic anemia in our population. Dietary insufficiency, poor absorption and increase demand might be contributing to high prevalence of vitamin B12 deficiency in our population. Therefore the medical community should seriously consider the merit of early screening for vitamin B12 deficiency and take precautions against the clinical consequences of vitamin B12 deficiency.

REFERENCES

[1] Aslinia F, Mazza J, Yale S. megaloblastic anemia and other causes of macrocytosis. Clin Med Res 2006; 4(3): 236-41. http://dx.doi.org/10.3121/cmr.4.3.236

[2] Wendy Brick, Russel Burgess-megaloblastic anemia and other causes of macrocytosis [Online]. 2006 [cited 2009 Aug 29]; Available from: URL: http://emedicine.medscape.com/article/203858-overview

[3] Rabhi S, Marroufi M, Khibri H, *et al*. Magnetic resonance imaging findings within the posterior and lateral columns of the spinal cord extended from the medulla oblongata to the thoracic spine in a woman with subacute combined degeneration without hematologic disorders. J Med Case Reports 2011; 5: 166. http://dx.doi.org/10.1186/1752-1947-5-166

[4] Iqbal SP, Kakepoto GN, Iqbal SP. Vit B12 deficiency-A major cause of megaloblastic anemia. J Ayub Med Coll Abbottabad 2009; 21(3): 92-4.

[5] Beutler E, Waalen J. The definition of anemia: what is the lower limit of normal of the blood hemoglobin concentration? Blood 2006; 107: 1747-50. http://dx.doi.org/10.1182/blood-2005-07-3046

[6] Patel KV, Harris TB, Faulhaber M, *et al*. Racial variation in the relationship of anemia with mortality and mobility disability among older adults. Blood 2007; 109: 4663-70. http://dx.doi.org/10.1182/blood-2006-10-055384

[7] Klee GG. Cobalamin and folate evaluation: measurement of methylmalonic acid and homocysteine vs vitamin B (12) and folate. Clin Chem 2000; 46(8 Pt 2): 1277-83.

[8] Andres E, Perrin AE, Kraemer JP, *et al*. Anemia caused by vitamin B 12 deficiency in subjects aged over 75 years: new hypotheses. A study of 20 cases. Rev Med Interne 2000; 21: 946-54.

[9] Hashim H, Tahir F. Frequency of vit B12 and folic acid deficiency among patients of megaloblastic anemia. Ann Pak Inst Med Sci 2006; 2(3): 192-4.

[10] Yakub M, Iqbal MP, Mehboobali N, *et al.* High prevalence of folic acid, vitamin B6 and vitamin B12 deficiencies is a predictor of hyperhomocysteinemia in a Pakistani urban population. In: Abstract Book: 21st International Medical Sciences Student Congress. Istanbul University. Istanbul 2008; 75.

[11] Khanduri U, Sharma A. Megaloblastic anemia: prevalence and causative factors. Natti Med J India 2007; 20(4): 172-5.

[12] Naeem MA, Uttra GM. Etiology of incidence of megaloblastic anemia in district Gilgit. Pak J Pathol 2007; 18(1): 15-6.

[13] Helicobacter pylori: A Cause of Vitamin B12 Deficiency World Applied Sciences Journal 2011; 12(9): 1378-81.

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http://dx.doi.org/10.6000/1927-5129.2012.08.02.68