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Original Article

A descriptive study of clinico-hematological profile of megaloblastic anemia in a tertiary care hospital

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ABSTRACT

Background: Megaloblastic Anemia (MA) is a relatively common disease, yet the data on prevalence of MA remains scarce. This study was conducted to study the prevalence and clinico-hematological profile of MA.

Methods: This was a cross-sectional study done on 1150 adult anemic patients. All patients diagnosed to have MA were studied for clinico-hematological and etiological profile. Nerve conduction studies (NCS) were done in all.

Results: MA was present in 3.6% cases of anemia. Severe anemia was seen in 9.7% of anemic patients and 75% of MA cases ($p < 0.05$). Forty five percent of MA patients presented with pancytopenia. Vitamin B12 and folic acid deficiency were documented in 40% and 25% cases respectively while combined deficiency was noted in 35% of all MA cases. There was no correlation between severity of anemia and deficiency of either of the vitamins (Fischer exact test: 0.530). Among MA patients, 35% were vegetarians while 65% consumed mixed diet. There was no association between vegetarian diet and Vit B12 deficiency ($p = 0.3137$). An additional etiology was more commonly found in patients on mixed diet [92%; 24/26] as compared to those on vegetarian diet [50%; 7/14] ($p = 0.04$). NCS was abnormal in 14 patients (35%). Overt clinical neuropathy was present in 12 cases of MA, while subclinical neuropathy was seen in 2 cases.

Conclusion: MA is one of common causes of pancytopenia and severe anemia. Diet plays an important role in causation of MA in vegetarians. An alternative etiology is however, more likely to be found in patients on mixed diet. While overt neurological abnormalities are common in MA, subclinical neuropathy is uncommon.

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Introduction

The prevalence of megaloblastic anemia (MA) reported by various Indian studies ranges from 02% to 40%.^{1–6} Most of these studies were carried out in children and in hospitalized patients. MA remains the commonest cause of macrocytic anemia⁷ and pancytopenia.^{8–12} It can have a varied clinical and hematological presentation.^{13–15} While overt neurological syndromes associated with MA are well documented, there is paucity of data on the existence of subclinical neurological manifestations in MA with one study published in British Journal in 1980 having shown a prevalence of 25%.¹⁶ In the current study, we calculated the prevalence of MA in adult patients of anemia, studied its clinico-hematological profile and the existence of overt and subclinical neuropathy in patients of MA.

Material and methods

We did a cross sectional study over a period of 18 months (Nov 2011–Apr 2013) at a tertiary care hospital of Maharashtra. All patients of anemia managed over a period of 18 months were evaluated. Pregnant and lactating women, children less than 12 years and the patients already on Vit B12/folic acid replacement or who have already been transfused were excluded. A written informed consent was obtained from all patients included in the study.

Hemogram was measured using 2 ml of ethylene diamine tetraacetic acid anticoagulated blood in automated Beckman Coulter 500 FC series analyzer. Anemia was defined as hemoglobin (Hb) < 13 g/dL in males and Hb < 12 g/dL in females, as per WHO definition of anemia. Severe anemia was defined as Hb < 8 g/dL. All patients of anemia with mean corpuscular volume (MCV) > 100 fL were evaluated with peripheral blood smear (PBS), bone marrow studies, Vit B12 and folic acid levels. Peripheral blood smear suggestive of MA was defined by presence of macro-ovalocytosis, anisocytosis, poikilocytosis, hypersegmented neutrophils and pancytopenia. The diagnosis of MA was confirmed with bone marrow aspiration studies where presence of hypercellular marrow with increased erythroid/myeloid ratio, megaloblasts, giant bands and metamyelocytes and decreased megakaryocytes favor the diagnosis of MA. Vit B12 and folic acid levels were measured using chemiluminescence method. Normal levels of Vit B12 and folic acid were defined as 211–911 pg/ml and >5.38 ng/ml respectively. A detailed dietary history was taken. Non vegetarian diet was defined as diet comprising predominately of animal food, mixed diet as one containing food of both plant and animal origin, vegetarian diet comprised of food of plant origin and milk, whereas pure vegan diet was defined as vegetarian diet less the milk. History of intake of drugs including proton pump inhibitor (PPIs) was taken along with history of previous surgery if any. All patients were asked about history of alcohol intake. Significant alcohol consumption was defined as more than 30 g of alcohol intake per day. History of neurological symptoms if any was noted. All patients were subjected to a detailed clinical examination.

Further evaluation of patients with MA included Liver Function Tests (LFTs), Lactate Dehydrogenase (LDH), Upper

Gastrointestinal Endoscopy (UGIE) and biopsy from stomach and duodenum for Histopathological Examination (HPE) besides the biopsies for *Helicobacter Pylori* (*H. Pylori*) detection by Rapid Urease Test (RUT). Additional tests like anti tissue transglutaminase antibodies (anti TTG antibodies), stool for cryptosporidiosis (for 03 days), Human Immunodeficiency Virus 1 and 2 (HIV 1 and 2) antibodies and colonoscopy were done if indicated. Parietal cell antibodies were looked for when there was evidence of atrophic gastritis or when there was suspicion of an auto-immune disease. Nerve Conduction Studies (NCS) were done in all patients of MA to document any subclinical neuropathy.

Statistical analysis: The continuous variables were analyzed using unpaired t test, while proportions and qualitative variables were analyzed using chi-square and Fisher's exact test. A 'p' value of less than or equal to 0.05 was considered statistically significant.

Results

A total of 1150 patients were diagnosed to have anemia, of which 59 patients were excluded as they have already been transfused or were on hematinics. Remaining 1091 (404 males and 687 females) patients were screened for macrocytosis (MCV > 100 fL). Macrocytosis was seen in 57/1091 patients. Three patients refused to give consent; the rest 54 patients were evaluated for MA with PBS, bone marrow studies, Vit B12 and folic acid levels. Forty patients (30 males and 10 females) were diagnosed to have MA (Fig. 1) which constituted 3.6% (95% CI: 2.5–4.7%) of all cases of anemia.

Mean age of MA patients was 36.15 ± 17.6 years which was significantly lower than mean age of all anemia cases (41.3 ± 15.3 years) ($p = 0.03$). Highest incidence of MA was noted in the age group between 16 and 20 years. Thirty-five percent (14/40) of MA patients and thirty six percent (397/1091) of total patients of anemia consumed vegetarian diet and there was no statistical difference between two cohorts ($p = 0.89$) as depicted in Table 1. Similarly, there was no statistical difference between significant alcohol consumption and regular PPI consumption between two groups ($p = 0.62$ and $p = 0.41$ respectively).

Symptoms and signs

Easy fatigability was the commonest symptom. One patient came with Congestive Cardiac Failure (CCF) due to anemia while two patients reported with acute Deep Vein Thrombosis (DVT) due to hyperhomocysteinemia secondary to Vit B12 deficiency. Thirty percent patients (12/40) presented with overt neurological symptoms and signs. While all twelve patients had evidence of peripheral neuropathy; two patients in addition presented with ataxia and had posterior column signs including positive Romberg's sign. Table 2 summarizes the symptoms and signs in our cohort of MA patients.

Investigations

Megaloblastic anemia (MA) constituted 3.4% (95% CI: 2.5–4.7%) of all cases of anemia. Severe anemia (Hb < 8 g/dL) was seen in

Fig. 1 – Consort diagram.

9.7% (106/1091) of all anemia cases and 75% (30/40) of MA cases ($p < 0.05$). Pancytopenia was noted in 45% (18/40) of MA patients. Hyper segmented neutrophils were noted in 57.5% (23/40) patients while ovalo-macrocytosis was documented in 45% (18/40) patients. Mean Hb noted in MA patients was 6.39 g/dL (± 1.95) and MCV recorded was 117.5 fL (± 5.082) with a mean red cell distribution width (RDW) of 21.53 (± 4.63). MCV > 110 fL and RDW ≥ 18 each correlated with a diagnosis of MA ($p = 0.001$). LDH activity was increased in all patients with MA. Mean LDH recorded in patient with MA was 3208 IU/L (range 589–9110 IU/L). Lower Hb level was associated with higher values of LDH. Indirect hyperbilirubinemia was seen in

50% (20/40) patients of MA; mean bilirubin recorded was 2.18 mg% (± 1.3).

Out of total 40 patients of MA, Vit B12 deficiency was noted in 40% (16/40) patients, folic acid deficiency in 25% (10/40) patients and 35% (14/40) patients had combined deficiency of Vit B12 and folic acid. There was no co-relation between the severity of anemia and vitamin B12 and/or folic acid levels, neither was there any co-relation between deficiency of a particular vitamin or combined deficiency and severity of anemia (Table 3).

Gastrointestinal investigations

Fourteen (14/40) patients tested positive for *Helicobacter Pylori* by RUT. Atrophic gastritis was reported in nine biopsies and parietal cell antibodies were done in all these patients. One out of these nine patients, had positive parietal cell and intrinsic factor antibody and was diagnosed to have pernicious anemia. Villous atrophy in duodenal biopsies was seen in 6/40 patients. TTG antibodies were detected in two patients with villous atrophy while four patients were diagnosed to have tropical sprue. Colonoscopy was done in three patients which was essentially normal.

Homocysteine levels were raised in two patients who presented with DVT; one of them had only Vit B12 deficiency while other had combined deficiency of Vit B12 and folic acid.

Diet and Megaloblastic Anemia (MA)

For analyzing the possible causative factors of MA, we divided the patients into two groups: Group 1 who consumed mixed diet (26/40) and Group 2 who consumed vegetarian diet (14/40). There were no vegans in our patients with MA. Combined Vit B12 and folic acid deficiency was found in 10 patients who consumed mixed diet and in 05 patients who consumed vegetarian food. Exclusive Vit B12 deficiency was found in 08 patients on vegetarian diet and in 09 patients on mixed diet while exclusive folic acid deficiency was noted in one vegetarian patient and in 07 patients on mixed diet. The possible etiological factors associated with MA seen in our study were *H. Pylori* infection (14/40;35%), chronic use of PPI (12/40;30%), atrophic gastritis (9/40;22.5%), significant alcohol consumption (8/40;20%), tropical sprue (4/40;10%), celiac sprue (2/40;5%), Acquired Immune Deficiency Syndrome (2/40;5%), pernicious anemia (1/40; 2.5%) and intestinal resection (1/40;2.5%). Out of 14 patients who were vegetarians, only 7 (50%) had one or more additional etiology whereas 24/26 (92.30%) patients who consumed mixed diet had one or more

Table 1 – Baseline characteristics.

	Megaloblastic anemia (40)	Anemia (1091)	p value
Males	30 (75%)	404 (37.03%)	0.001
Females	10 (25%)	687 (62.97%)	0.001
Mean age (yrs)	36.15 $\pm$ 17.6	41.3 $\pm$ 15.3	0.03
Vegetarian diet	14 (35%)	397 (36.38%)	0.89
Mixed diet	26 (65%)	694 (63.61%)	0.76
Alcohol consumption (>30 g/day)	8 (20%)	196 (17.96%)	0.62
Regular Proton Pump inhibitor consumption	10 (25%)	345 (31.62)	0.41

Table 2 – Symptoms and signs in megaloblastic anemia.

Symptoms n (%)		Signs n (%)	
Easy fatigability	35 (87.5)	Pallor	37 (92.5)
Anorexia	28 (70)	Bald tongue	26 (65)
Breathlessness on exertion	24 (60)	Glossitis	17 (42.5)
Palpitations	19 (47.5)	Knuckle pigmentation	15 (37.5)
Weight loss	16 (40)	Jaundice	15 (37.5)
Oral ulcers	12 (30)	Angular cheilitis	14 (35)
Paresthesias and gait instability	12 (35)	Hepatomegaly	13 (32.5)
Fever	5 (12.5)	Hair changes	10 (25)
Diarrhea and vomiting	6 (15)	Pedal edema	6 (15)
DVT	2 (5)	Splenomegaly	5 (12.5)
DVT, deep vein thrombosis.			

Table 3 – Co-relation between deficiency of a particular vitamin or combined deficiency and severity of anemia.

	Deficiency of both Vit B12 and folic acid	Only Vit B12 deficiency	Only folic acid deficiency
Moderate anemia	4	5	1
Severe anemia	10	11	9
Fischer's exact test value: 0.530.			

Table 4 – Association between diet and type of vitamin deficiency.

	Total Vit B12 def (only Vit B12 deficiency + combined Vit B12 and folic acid deficiency)	Only folic acid def
Mixed diet	19	7
Vegetarians	13	1
Chisquare test: 0.3137 (no significant association between diet and type of vitamin deficiency).		

than one etiology. The etiological work up was more likely to be negative in vegetarians (fisher's exact test value: 0.04). This implies that diet plays an important role in causation of MA in vegetarians. There was no statistical association between type of diet and deficiency of particular vitamin as depicted in Table 4.

Nerve Conduction Studies (NCS)

Twelve patients (12/40) had clinical evidence of large sensory fiber involvement which was confirmed by NCS. Apart from these, two patients had evidence of subclinical neuropathy (demyelinating type) as demonstrated by delayed latencies on NCS. There was no co-relation between Hb levels and neurological findings clinically or on NCS. Out of 14 patients who had clinical and/or electrophysiological neurological abnormalities, 50% (7/14) had deficiency of Vit B12 alone; 28.57%(4/14) had deficiency of folic acid alone and 21.43% (3/14) had deficiency of both vitamin B12 and folic acid.

Discussion

Most of the studies on prevalence of MA have been done in children and the prevalence of MA in these studies have been documented anywhere between 2% and 42%.^{1–5,17} The prevalence of MA in our adult patients of anemia was 3.6%. Most of our patients of MA were males (30/40). This observation may be due to the fact that females tend to be more iron deficient and in presence of iron deficiency, the MCV won't rise above 100 fL even with co-existent Vit B12 or folic acid deficiency. We could

not do the iron profile of our whole anemia cohort because of financial constraints and also as it was beyond the scope of our study. The male preponderance for MA has been noted in another study by Maktouf et al.¹⁸ While studies have shown an increased prevalence of MA in vegetarians,^{19,20} majority of our patients consumed mixed diet (65%) and none of them was a pure vegan. Though the limitation of our finding was that the number of MA patients was small; but still we would like to point out an important observation that diet does seem to play a role, as the etiological work up was more likely to be negative in vegetarian group (Fischer's exact test value: 0.04). There was no significant association between diet and type of vitamin deficiency (Chisquare test: 0.3137). RUT for *Helicobacter Pylori* was positive in 12/26 patients (35%). This organism has been postulated to play a role in causing MA in many studies^{21–24}; though causal role could not be established. History of chronic intake of PPI was given by 12/40 (30%) patients. These drugs may play a role in malabsorption of cobalamin.^{25,26} Whether patients take PPI to ameliorate the mucosal effects of megaloblastic anemia or the use of PPI lead to megaloblastosis is not clear.¹⁷

Vitamin B12 deficiency was found in 40% of our patients, folic acid deficiency was seen in 25% while combined deficiency was recorded in 35% patients. The Indian series from 1960s documented folate deficiency to be more common cause of MA.^{27,28} Subsequent studies done in 1980s and 1990s highlighted that Vit B12 deficiency is far more common than folate deficiency.^{5,29–32} Increased prevalence of Vit B12 deficiency as compared to folic acid deficiency has been reported from countries outside India also.^{33–36} Indirect hyperbilirubinemia and raised LDH are commonly found in

MA which occur due to ineffective erythropoiesis. Lower Hb level was associated with higher values of LDH as has also been noticed by Emerson et al.³⁷

In our study, MA accounted for 28.30% of all cases of severe anemia. This has also been shown in another study where MA accounted for 42.5% of all cases of severe anemia.³⁸ In our study, 01 patient presented with CCF, 02 patients had DVT on presentation, 02 patients presented with ataxia, while 05 patients were admitted as case of fever with hepatomegaly and/or hepatosplenomegaly. As many as 18 patients presented with pancytopenia. Such varied presentations of MA have been documented in earlier studies also.^{8–12,15,39–44} MA can mimic aplastic anemia, leukemia and myelodysplastic syndrome. Even an expert hemato-pathologist may find it difficult to differentiate between myelodysplastic syndrome and megaloblastosis on bone marrow without the support of cytogenetics. Overt neurological manifestations were found in 30% patients as has been documented in the literature earlier.¹⁶ The sub-clinical neurological manifestations were documented only in 02 patients unlike the previous report where a prevalence of 25% was reported. There was no relation between the neurological manifestations and the Hb levels as was demonstrated in other studies also.⁴⁵

Conclusions

Prevalence of MA in adult patients with anemia was found to be 3.6%. MA is one of the common causes of severe anemia and constituted 28.30% of all causes of severe anemia. MA is not restricted to vegetarians only and is also seen quite commonly in those who consume non vegetarian food. However, the people who take vegetarian diet are more likely to have negative etiology thus emphasizing the role of diet in MA patients. The presentation of MA can be varied. While overt neurological abnormalities are common in MA, subclinical neuropathy is uncommon.

Limitations

The prevalence of MA in cohort of anemia may be a little under-estimation as we did not evaluate the patients whose MCV was less than 100 fL, so we could have missed MA when it co-existed with iron deficiency or with other causes of microcytic anemia. It was a cross sectional study so we could not establish the causal relationship to various etiological factors studied.

Conflicts of interest

The authors have none to declare.

REFERENCES

- Ghai OP. Aetiology of anemias in infancy and childhood (in New Delhi). *Indian J Child Health*. 1956;95–100.
- Dalal RJ, Udani PM, Parekh JG. Megaloblastic anemia in infancy and childhood. *Indian J Pediatr*. 1969;6:255–262.
- Ghai OP, Choudhry VP, Singla PN, Saraya AK. Nutritional macrocytic anemia in infancy and childhood. In: Ghai OP, ed. *New Developments in Pediatric Research*. New Delhi: Interprint; 1977:121.
- Sharma A, Sharma SK, Grover AK, Tewari AD, Abrol P. Anemia in protein energy malnutrition. *Indian Pediatr*. 1985;22:841–844.
- Gomber S, Kumar S, Rusia U, Gupta P, Agarwal KN, Sharma S. Prevalence and etiology of nutritional anemias in early childhood in an urban slum. *Indian J Med Res*. 1998;107:269–273.
- Gera R, Singh ZN, Chaudhury P. Profile of nutritional anemia in hospitalized children over a decade. In: *Conference Abstracts, 38th National conference of Indian Academy of Pediatrics Patna, HO-09*. 2001; 60.
- Unnikrishnan V, Kumar Dutta T, Badhe BA, Bobby Z, Panigrahi AK. Clinico-aetiological profile of macrocytic anemias with special reference to megaloblastic anemia. *Indian J Hematol Blood Transfus*. 2008;24(4):155–165.
- Santra G, Das BK. A cross-sectional study of the clinical profile and aetiological spectrum of pancytopenia in a tertiary care centre. *Singapore Med J*. 2010;51(10):806–812.
- Premkumar M, Gupta N, Singh T, Velpandian T. Cobalamin and Folic Acid status in relation to the etiopathogenesis of pancytopenia in adults at a tertiary care centre in North India. *Anemia*. 2011;2012:1–12.
- Bhatnagar SK, Chandra J, Narayan S, Sharma S, Singh V, Dutta AK. Pancytopenia in children – etiological profile. *J Trop Pediatr*. 2005;51:296–299.
- Khunger JM, Arulselvi S, Sharma U, Ranga S, Talib VH. Pancytopenia – a clinicohematological study of 200 cases. *Indian J Pathol Microbiol*. 2002;45:375–379.
- Kumar R, Kalra SP, Kumar H, Anand AC, Madan H. Pancytopenia – a six year study. *J Assoc Physicians India*. 2001;49:1078–1081.
- Zengin E, Sarper N, CakiKilic S. Clinical manifestations of infants with nutritional vitamin B12 deficiency due to maternal dietary deficiency. *Acta Pediatr*. 2009;98:98–102.
- Chandra J. Megaloblastic anemia: back in focus. *Indian J Pediatr*. 2010;77:795–799.
- Marwaha RK, Singh S, Garewal G, Marwaha N, Walia BNS, Kumar L. Bleeding manifestations in megaloblastic anemia. *Indian J Pediatr*. 1989;56:243–247.
- Shorvon SD, Carney MWP, Chanarin I, Reynolds EH. The neuropsychiatry of megaloblastic anaemia. *BMJ*. 1980;281:1036–1038.
- Khanduri U, Sharma A. Megaloblastic anemia: prevalence and causative factors. *Natl Med J India*. 2007;20(4):172–175.
- Maktouf C, Bchir F, Louzir H, et al. Megaloblastic anemia in North Africa. *Haematologica*. 2006;91:990–991.
- Chanarin I, Malkowska V, O'Hea AM, Rinsler MG, Price AB. Megaloblastic anemia in vegetarian Hindu community. *Lancet*. 1985;42:1168–1172.
- Carmel R. Nutritional vitamin B12 deficiency: possible contributory role of subtle vitamin B12 malabsorption. *Ann Intern Med*. 1978;88:647–649.
- Annibale B, Lahner E, Bordini C, et al. Role of H pylori infection in pernicious anemia. *Dig Liver Dis*. 2000;32:756–762.
- Perez-Perez GI, Blaser MJ. Role of helicobacter pylori infection in the development of pernicious anemia. *Clin Infect Dis*. 1997;25:1020–1022.
- Andres E, Loukili NH, Noel E, et al. Vitamin B12 (cobalamin) deficiency in elderly patients. *CMAJ*. 2004;171:251–259.
- Laine L, Lewin D, Naritoku W, Estrada R, Cohen H. Prospective comparison of commercially-available rapid urease tests for the diagnosis of Helicobacter pylori. *Gastrointest Endosc*. 1996;44:523.

25. Marcuard SP, Albernaz I, Khazanie PG. Omeprazole therapy causes malabsorption of cyanocobalamin (vitamin B12). *Ann Intern Med.* 1994;120:211–215.
26. Schenk BE, Kuipers EJ, Klinkenberg-Knol EC, et al. Atrophic gastritis during long-term omeprazole therapy affects serum vitamin B12 levels. *Aliment Pharmacol Therap.* 1999;13:1343–1346.
27. Bhende YM. Some experience with nutritional megaloblastic anemia. *J Postgrad Med.* 1965;11:145–155.
28. Mittal VS, Agarwal KN. Observations on nutritional megaloblastic anemia in early childhood. *Ind J Med Res.* 1969;57:730–738.
29. Sarode R, Garewal G, Marwaha N, et al. Pancytopenia in nutritional megaloblastic anemia: a study from north-west India. *Trop Geogr Med.* 1989;41:331–336.
30. Gomber S, Kela K, Dhingra N. Clinico-hematological profile of megaloblastic anemia. *Indian Pediatr.* 1998;35:54–57.
31. Chandra J, Jain V, Narayan S, et al. Folate and cobalamin deficiency in megaloblastic anemia in children. *Indian Pediatr.* 2002;39:453–457.
32. Khanduri U, Sharma A, Joshi A. Occult cobalamin and folate deficiency in Indians. *Natl Med J India.* 2005;18:182–183.
33. Mukibi JM, Makumbi FA, Gwanzura C. Megaloblastic anemia in Zimbabwe: spectrum of clinical and hematological manifestations. *East Afr Med J.* 1992;9:83–87.
34. Madood-ul-Mannan. Anwar M, Saleem M. Study of serum vitamin B12 and folate levels in patients of megaloblastic anemia in northern Pakistan. *J Pak Med Assoc.* 1995;45:187–188.
35. Allen LH, Rosado JL, Casterline JE, et al. Vitamin B12 deficiency and malabsorption are highly prevalent in Mexican communities. *Am J Clin Nutr.* 1995;65:1013–1019.
36. Casterline JE, Allen LH, Ruel MT. Vitamin B12 deficiency is very prevalent in lactating Guatemalan women and their infants at three months postpartum. *J Nutr.* 1997;127:1966–1972.
37. Emerson PN, Wilkinson JH. Lactate dehydrogenase in the diagnosis and assessment of response to treatment of megaloblastic anemia. *Br J Haematol.* 1966;12:678–688.
38. Patra S, Pemde HK, Singh V, Chandra J, Dutta A. Profile of adolescents with severe anemia admitted in a tertiary care hospital in northern India. *Indian J Pediatr.* 2011;78(7):863–865.
39. Wald DS, Law M, Morris JK. Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. *BMJ.* 2002;325:1202–1206.
40. Clarke R, Collins R, Lewington S, et al. Homocysteine and risk of ischemic heart disease and stroke: a meta analysis. *JAMA.* 2002;288:2015–2022.
41. Lonn E, Yusuf S, Arnold MJ, et al. Homocysteine lowering with folic acid and B vitamins in vascular disease. *N Engl J Med.* 2006;354(15):1567–1577.
42. Tilak V, Jain R. Pancytopenia: a clinico-haematologic analysis of 77 cases. *Indian J Pathol Microbiol.* 1999;42(4):399–404.
43. Lam S, Lam BL. Bilateral retinal hemorrhages from megaloblastic anemia: case report and review of literature. *Ann Ophthalmol.* 1992;24(3):86–90.
44. Davies JK, Guinan EC. An update on the management of severe idiopathic aplastic anemia in children. *Br J Hematol.* 2007;136:549–564.
45. Briani C, Dalla Torre C, Citton V, et al. Cobalamin deficiency: clinical picture and radiological findings. *Nutrients.* 2013;5(11):4521–4539.