ORIGINAL ARTICLE

Importance of PLT/MCH Ratioto Differentiate Isolated B12 Deficiency From B12 Deficiency Combinedwith Other Causes

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ABSTRACT

Objective: To explore the importance of platelet/mean corpuscular haemoglobin ratio to distinguish between isolated B12 deficiency from combine B12 with iron or B12 with beta thalassaemia trait

Methodology: Two types of cases were selected including isolated B12 deficiency and B12 deficiency combined with iron deficiency or beta thalassemia trait. A total of 105 cases were included. Complete blood counts were done including red cell indices, serum ferritin and Hb electrophoresis. Two arms were compared with each other with a p value less than 0.05 considered as significant.

Results: Mixed deficiencies group of B12 showed decreased haemoglobin level, mean corpuscular volume, mean corpuscular haemoglobin,and mean corpuscular haemoglobin concentration and increased platelet count as compared to solo B12 deficient group. CBC indices indicated that PLT/MCH ratio parameter was higher in value in combined deficiencies as compared to B12 deficiency alone.

Conclusion: It was concluded that with the help of Platelet/mean corpuscular haemoglobin ratio and hemogram indices, we can distinguish simple B12 deficiency from complicated B12 with iron deficiency or beta thalassaemia trait on the basis of complete blood count. Physicians should keep in mind these indices and ratio to distinguish mixed deficiencies on routine blood count thus initiating timely further management.

Key words: Vitamin B12, CBC indices, PLT/MCH ratio and MCV

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عنوان: حیاتین B12 کی جداگانہ کمی اور دوسری وجوہات کی وجہ سے اس کی کمی کے درمیان امتیاز کرنے کے لیے PLT/MCH تناسب کی اہمیت۔

مقصد: اس مطالعہ کا مقصد پلیٹلیٹس اور اوسط ہیموگلوبن کارپوسکیولر کے PLT/MCH تناسب کی مدد سے حیاتین B12 کی جداگانہ کمی اور دوسری مشترکہ وجوہات (B12 اور آئرن اور بیٹا تھلیسیما) کی وجہ سے کمی کے درمیان فرق کو جانچنا ہے۔

طریقہ: حیاتین B12 کی کمی کے تین مختلف قسم کے کیسز بشمول حیاتین B12 کی جداگانہ کمی اور دوسری مشترکہ وجوہات (B12 اور آئرن اور بیٹا تھلیسیما) اس مطالعہ کے لیے منتخب کی گئیں۔ ہر قسم کے لیے مجموعی طور پر 105 مریضوں کو شامل کیا گیا۔ اور انکے خون کے نمونے لے کر CBC کا ٹیسٹ کیا گیا جس میں سرخ خلیات، سیرم فیریٹین اور Hb الیکٹروفورسس کی جانچ کی گئی۔ حاصل ہونے والے نتائج کو SPSS ورژن 22 پر شماریاتی تجزیہ کے لیے جانچنے۔ اور تینوں اقسام کا موازنہ کیا۔ (p-value=0.05)

نتائج: حاصل ہونے والے نتائج سے معلوم ہوا کہ مشترکہ اقسام میں حیاتین B12 کی کمی کی وجہ سے ہیموگلوبن لیول، MCV، MCH، MCHC اور پلیٹلیٹس کی تعداد میں خالی B12 کی کمی والے کیسز کے مقابلے میں کمی ریکارڈ کی گئی۔ CBC کے حاصل ہونیوالے نتائج سے PLT/MCH تناسب نکالنے پر معلوم ہوا کہ تناسب کی قیمت حیاتین B12 جداگانہ کمی کے مقابلے میں دوسری مشترکہ وجوہات (B12 اور آئرن اور بیٹا تھلیسیمیا) میں زیادہ پائی گئی۔

حاصلِ مطالعہ: حاصل شدہ نتائج سے یہ بات اخذ کی جاتی ہے کہ PLT/MCH تناسب اور ہیموگرام کے نتائج سے ہم B12 کی سادہ اور پیچیدہ (آئرن کی کمی اور بیٹا تھلیسیمیا) کمی کے درمیان واضح فرق CBC کی بنیاد پر کر سکتے ہیں۔ اور اس بات کی تجویز کی جاتی ہے کہ طبیب CBC کی جانچ کہ وقت ان دو چیزوں کو ذہن میں رکھیں۔

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INTRODUCTION

Deficiency of vitamin B12 is prevalent in this part of the world due to various reasons. Prompt and timely identification of this disorder is of utmost clinical importance[1].Mixed deficiencies are underestimated globally due to lack of awareness, non-availability and high cost of investigations. According to the global nutritional report in International Conference on Nutrition (ICN2), Pakistan has still not recovered from malnutrition and anaemia especially in children under five years and women of child bearing age[2].

According to the National Nutrition Survey 2018, 60% of Pakistani mothers and children experienced micronutrient deficiency, of whom 51% pregnant women were anaemic and 37% suffered from iron deficiency anaemia. Significant percentage of children (61.9%) reported anaemia out of whom 43.8% suffered from iron deficiency anaemia. In short, 50% of Pakistani women of reproductive age group are anaemic[3]. Children of these malnourished and deficient mothers receive impact on their mental and physical growth. These children became stunted, have low weight or height for age or may have cognitive deficits which in turn burden the families with recurrent infections or poor health issues.

Identifying or predicting the mixed deficiencies or underlying cause on hemogram indices is very beneficial for our population, which suffers from multiple micronutrient deficiencies and generally cannot afford other expensive investigations. Commonly, physicians miss the diagnosis of mixed deficiencies due to lack of certain diagnostic morphological features. At times, a diagnosis is reached only when the patients develop typical features of advanced severe deficiency, for example neurological manifestations in case of B12 deficiency along with other deficiencies[4]. We agree that certain diagnostic features are usually associated with individual vitamin or mineral deficiency or genetic disorder but every time this should not be relied upon. As in some situations, these morphological features are masked due to some other confounding factors or it might be possible that patients have any chronic illness which affects the morphology[5]. This practise is very common in Pakistan which could impair the identification of several cases[6].

Literature proves that iron deficiency is identified to be the most frequent nutritional deficiency in Pakistan. The country has still not achieved its goal of overcoming iron deficiency among susceptible individuals which requires high iron intake[7-9]. According to the report by UNICEF, the prevalence of iron deficiency in Pakistan in children less than five years old is 56%[10]. In Pakistan, each year 50% of deaths in children less than 5 years occur due to under nourishment[11,12]. In urban slums of Karachi, the prevalence of iron deficiency anaemia in children aged 6-60 months was 61%[13] which is a high figure.A study in 2007 reported high frequency of anaemia (78.7%) in rural based preschool children of Karachi[14]. In Pakistani children, impaired cognition, growth retardation, and reduced physical activity due to iron deficiency has been widely documented[15,16]. As reported by World Health Organization surveys, iron deficiency anaemia is a severe health issue in preschool children followed by the same in pregnant and non-pregnant women of reproductive age in Pakistan[17].

Beta thalassemia is the most common health problem in Pakistan[18]. The word was introduced in countries adjoining the Mediterranean Sea because this disease is more common in people of the Mediterranean region, the Indian Subcontinent and in the Middle East. First explained by Cooley and Lee in 1925[19], it is the most widespread heritably transmitted blood disorder with a carrier rate of 5-8% in different ethnic groups all over the country roughly seven to ten million individuals. This blood disorder is due to decreased beta globin chain synthesis usually due to mutations in beta globin genes. Approximately greater than 200 different genetic defects have been identified to date[18].

Screening of population, proper genetic counselling, pre-natal screening, discouraging inter family marriages in such cases and termination of pregnancies with thalassaemia major fetus are strategies that can reduce the burden of disease[19].

When thalassaemia is associated with concomitant micronutrient and vitamin deficiencies, the severity of anaemia is enhanced[20,21]. On haematological examination, uncomplicated anaemia can be easily diagnosed with a characteristic feature of its disease. However, at times these morphological features can be masked by related conditions for example B12 deficiency or iron deficiency as reported earlier by different authors[22-25].

Therefore, in this research we emphasize on parameters of complete blood count and platelet/mean corpuscular haemoglobin ratio to distinguish complicated cases of mixed deficiencies from simple B12 deficiency. Furthermore, in routine clinical practice, on the basis of these parameters, further workup or baseline investigation of these cases should be done in order to avoid permanent complications as prevention is better than cure.

METHODOLOGY

Research was carried out in the department of Dr. Ishrat-ul-Ebad Khan Institute of Blood Diseases, Dow

University of Health Sciences, Karachi, during the period of January 2014 to September 2014. Total 105 B_{12} deficient cases (B_{12} <200ng/l) with (MCV <95/fl) were recruited in this study. Parameters including complete blood count, serum ferritin and Hb electrophoresis were done on all samples. A sample size of 105 was estimated using open epi version 3.01, open source calculator with 7.35% prevalence of iron deficiency or thalassaemia trait in B_{12} deficient patients at 95% confidence interval[5]. Samples were studied into two groups. First group consist of 63 cases who had B_{12} deficiency only, remaining 42 cases with combined B_{12}, iron deficiency and beta thalassaemia trait. Complete blood count parameters of two groups were compared with each other with a p-value less than 0.05 considered as significant.

Complete Blood Count was performed on CELL DYN 3200 multiparameter automated haematology analyzer by using flow cytometric technique. This technique analyzes the red blood cells,white blood cells, and platelets. It is a process in which individual cells or other biological particles in a single file produced by a fluid stream are passed through a beam of light. A sensor measures the physical or chemical characteristics of the cells or particles by the loss or scattering of light. Flow cytometry enables the rapid screening of large numbers of cell analysis at the single cell level. Meanwhile, peripheral smears were also made and stained by using Leishman stain to assess morphology. On the same samples, serum ferritin was performed on Immulite 1000 analyzer by using solid phase competitive method. It involves a competition binding between the labeled antigens and non-labeled antigens to the limited amount of antibody binding sites.

Haemoglobin electrophoresis was done on genios instrument at alkaline pH by using principle involved normal or variant haemoglobins showed different electrophoretic mobility and recognized by zone electrophoresis performed on cellulose acetate.

Table 1: Haemogram Indices of Total Study Population

Haemogram Indices	Total study population (n=105)		
Variables	Minimum value	Mean± SD	Maximum value
Hb (gm/dl)	2.5	12.40±2.61	15.4
RBC(10E12/L)	1.3	4.93±0.88	6.7
MCV (fl)	53	79.34±9.46	94
MCH(pg)	14	25.3±3.76	31
MCHC(%)	27	31.65±1.71	35
TLC(10E9/L)	3.0	7.77±1.93	14.8
Platelets(10E9/L)	52	295.36±99.445	718

Hb: Haemoglobin MCH: Mean Corpuscular Haemoglobin
RBC: Red Blood Cells MCHC: Mean Corpuscular Haemoglobin
MCV: Mean Corpuscular Volume TLC: Total Leukocyte Count
SD: Standard Deviation

Table 2: Comparison of Haemogram Indices Between B12 Deficient Cases With Iron Deficiency or Thalassaemia Trait and Without Iron Deficiency or Thalassaemia Trait

Variables	B12 with iron deficiency or thalassaemia trait			Isolated B12 deficiency			Independent sample T test P- Value
	Min. value	Mean ± SD	Max. Value	Min. value	Mean± SD	Max. Value	
Hb	2.5	10.89±2.42	15.2	5.86	13.29±2.33	15.4	* <0.01
RBC	1.3	4.98±1.04	6.7	2.05	4.91±0.74	6.4	<0.68
MCV	53	71.89±10.46	89	71	83.81±5.58	94	*<0.01
MCH	14	22.31±4.17	30	20	27.07±2.04	31	*<0.01
MCHC	27	30.80±1.75	34	28	32.16±1.50	35	*<0.01
TLC	5.5	8.31±2.17	14.8	3.0	7.51±1.87	12.7	*<0.04
Platelets	52	316.49±1.3.03	580	124	282.68±94.97	586	<0.08
PLT/MCH Ratio	14.18			10.44			

*P < 0.05 considered significant using independent sample t-test
Hb: Haemoglobin MCH: Mean Corpuscular Haemoglobin
RBC: Red Blood Cells MCHC: Mean Corpuscular Haemoglobin
MCV: Mean Corpuscular Volume TLC: Total Leukocyte Count
SD: Standard Deviation

DISCUSSION

In Pakistan, cases of mixed micronutrient deficiencies are very common[6]. Prevalence of these mixed deficiencies could be even higher than reported in Pakistani population. Sometimes identification of these cases becomes very difficult because these deficiencies may mask each other's morphological features and treatment of one may reveal the deficiency of the other. On the basis of haemogram indices, we can recognize these mixed deficiencies timely to some extent.

Our findings are in close accordance with a study by Beyan C etal in Turkey who compared haematological parameters of IDA group alone with IDA-B_{12} deficient group and from that group IDA-B_{12} cases had more decreased values of Hb, MCV, MCH and MCHC[1]. Similar findings were reported by Jolobe and Chan C.W.J. et./al[5,26]. It could be due to combine B_{12}-IDA deficiency or concurrent beta thalassaemia trait which causes more impediment in the maturation of erythroid progenitors in immature stages and decreased Hb synthesis.

On the other hand, our study showed increased platelet count in combined deficiencies as compared to B_{12} deficiency alone. We can speculate that high platelet count in combined deficiencies was due to iron deficiency. However, we also suggest PLT/MCH ratio for differentiating B_{12} deficiency alone from combine B_{12}-Iron in which PLT/MCH ratio becomes high. This finding is also consistent with the finding of Beyan C et al who recommended PLT/MCH ratio to screen mixed deficiency of iron and B_{12}. He reported high PLT/MCH in IDA-B_{12} group as compared to IDA alone and recommended cut off value as >12 . The most convenient cut off value of PLT/MCH ratio screening is >12 which has 74.6% sensitivity and 41.9% specificity[1]. On the basis of this parameter, cases with high PLT/MCH ratio should be recommended for further investigations.

It is an established fact that iron deficiency aneamia may cause thrombocytosis. Research from Turkey compared thrombopoietic cytokines 6 in iron deficient women with thrombocytosis in iron deficient women with normal platelet count and reported that thrombopoietin, leukemia inhibitory factor, interleukin-6 and interleukin-11 levels were normal except erythropoietin and concluded that erythropoietin increases platelet count[27]. Another study suggested that in moderate iron deficiency anaemia, the cause of thrombocytosis may be due to multiple reasons such as entry of large numbers of parent cells in the megakaryocyte section with increasing outflow; secondly, reduced megakaryocyte maturation; thirdly, shunt of stem cells and triggering effect of transferrin on platelet production; and last one is inhibitory effect of iron on megakaryocyte maturation[28].

A recent case report showed 32 year old female presenting with paraparesis. Her complete blood count parameters showed hypochromia, microcytosis and increased red cell distribution width. Physicians considered conditions like Guillain Barre syndrome, multiple sclerosis, diabetic neuropathy, and major depression. Initially, she was treated for Guillain Barre syndrome. Further investigations revealed B_{12} deficiency with beta thalassaemia trait. Upper gastrointestinal endoscopy revealed pernicious anaemia and patient immediately responded to intravenous (B_{12}) methylcobalamin for ten days. Elements in this case which misled the physician were microcytosis and absence of anaemia with paraparesis[29].

A study in India reported a case of thalassaemic intermediate child presenting with acute flaccid paralysis. His haemoglobin was 6.1 gm/dl and peripheral smear showed microcytic blood picture with evidence of haemolysis. Further work up revealed severe B_{12} deficiency[30]. In another case report from Croatia, a 16-year-old beta thalassaemic trait girl presented with paraparesis and paresthesias. Patient and her family were on complete vegetarian diet. Her B_{12} was low. Peripheral smear showed anisocytosis and hypersegmented neutrophils[31]. Thus it is suggested that B_{12} deficiency with beta thalassaemia trait can present with or without anaemia. Therefore, physicians must not overlook these types of cases and mixed deficiencies or heritably transmitted disorders should be included in the list of differential diagnosis.

The underlying pathophysiology of beta thalassaemia trait is reduced beta globin chain production. On the other hand, the synthesis of alpha globin chain remains unaltered. This disproportion leads to excess alpha chains. These free excess alpha chains are very unstable and precipitate in red cell precursors forming inclusions that hamper the maturation of red blood cells.

Another case is from Korea of an 18-year-old young male patient admitted with severe anaemia, short stature, and delayed puberty. His complete blood count showed haemoglobin 3.3g/dl. Reticulocyte count were 0.59% and red cell distribution width was 22.2%. Peripheral blood smear showed hypersegmented neutrophils and normocytic hypochromic red blood cells. Patient had a history of small bowel resection from 50 cm below the treitz ligament to 5cm above the ileocecal valve. Differential diagnosis included nutritional anaemia, chronic renal failure, inflammatory bowel disease or inherited anaemia but the patient responded dramatically

on B_{12} and iron replacement therapy. Retic count became 14.9% on the sixth day of treatment[32]. Therefore, cases with resection of small intestine should be carefully observed for nutritional deficiencies. We observed anisopoikilocytosis and hypochromasia in 37.14% and 30.4% cases respectively, showed RDW from 16% to 23% which is very close to a study that also reported 32% anisopoikilocytosis and 26.3% hypochromasia which is usually present in combined deficiencies[5]. We took a detailed nutritional history about consumption of animal food especially beef, mutton, and dairy products which are the main sources of vitamin B_{12} and iron. According to the history, 65% patients belonged to moderately poor socioeconomic group. Despite the majority of our population being non vegetarian, probably poverty restricted the accessibility and consumption of foods of animal origin. We observed that 47.6% patients consumed meat less than twice per month.

In this study, by comparing CBC parameters of combined deficiencies and B_{12} deficiency alone, we found that in combined deficiencies there were low Hb, MCH, MCHC, and MCV while high platelets, PLT/MCH ratio, and RDW which are helpful in differentiating pure B_{12} from IDA-B_{12} cases to some extent.

CONCLUSION

Study concludes that morphological features of mixed deficiency cases are not as diagnostic as in isolated deficiency. With the help of simple platelet/mean corpuscular haemoglobin ratio, the physicians can distinguish simple B_{12} from combined B_{12} deficiencies on complete blood count.

Authors' contributions: Dr Asma Shaikh conceived the study, searched for literature, contributed in data collection, analysis and review, and worked on introduction and discussion. Dr Suresh Kumar and Dr Zareen Irshad worked on results and discussion. Dr Nadeem Nusrat and Dr Salma Perveen reviewed the literature, contributed to the discussion and edited the manuscript. Dr Sadaf Razzak and Prof Mehmood Hasan reviewed the literature, results and conclusion. All authors contributed to the final manuscript.

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