IRON DEFICIENCY AND THALASSAEMIA; IRON DEFICIENCY AND THALASSAEMIA TRAIT IN VITAMIN B12 DEFICIENT PATIENTS WITH NORMAL OR LOW MEAN CORPUSCULAR VOLUME

Asma Shaikh¹, Nadeem Nusrat², Muhammad Akbar Agha³, Asma Shabbir⁴

ABSTRACT... Objectives: To study the importance of normal or low mean corpuscular volume in vitamin B12 deficiency due to co-existence of iron deficiency or beta thalassaemia trait masking a rise in mean corpuscular volume. Study Design: Observational non-probability cross sectional study. Setting: DDRRL. Period: January 2014 to September 2014. Methods: 105 vitamin B12 deficient cases (vitamin B12 less than 200ng/l) who presented with normal or low mean corpuscular volume (MCV less than 95 fl) on complete blood count (CBC) were determined from Dow diagnostic research and reference laboratory (DDRRL). Serum ferritin, red blood cell folate (RBC Folate) level and Hemoglobin electrophoresis for beta thalassaemia trait were analysed in these patients. Results: Total of 105 vitamin B12 deficient patients who fulfilled the inclusion criteria were enrolled in this study from which 39 (37.14%) were male and 66 (62.85%) were females. Amongst them 36.19% had microcytosis with the mean age of 37±16.2 years while 63.8% were normocytic with mean age of 41.58±15.65 years. In microcytic group, iron deficiency, beta thalassaemia trait, combined deficiency of B12, iron and beta thalassaemia trait and RBC folate deficient were 52.6%, 34.21%, 7.8% and 2.63% respectively. In normocytic group, iron deficiency, beta thalassaemia trait and RBC Folate deficient were 13.4%, 00% and 11.9% respectively. Conclusion: There is a significant coexistent frequency of iron deficiency and beta thalassaemia trait in vitamin B12 deficiency with normal or low MCV especially in females of 20-40 years of age. There should be a high index of suspicion for B12 deficiency when investigating anaemia with normal or altered red cell indices.

Key words: Vitamin B12, MCV, Iron Deficiency, Beta Thalassaemia Trait, Ferritin, RBC Folate, Microcytic and Normocytic Anaemia.

INTRODUCTION

Vitamin B12 is one of the most important vitamin of the B-complex group. Its deficiency has systemic effects with involvement of nearly all body systems and if not detected and treated in time, it may lead to significant morbidity or even mortality directly or indirectly.¹ Recent researches have suggested that the incidence and prevalence of vitamin B12 deficiency is far more than estimated at clinical and sub-clinical levels.² Deficiency of vitamin B12 is under estimated globally due to lack of awareness, non-availability of resources and high cost of the investigations.³ ⁴ Its deficiency is not always accompanied by macrocytic picture refracted in the form of raised MCV. This rise in MCV can be blunted by concomitant iron deficiency or thalassaemia trait. Due to these coexistent conditions, B12 deficiency can be missed on routine CBC leading to development of severe complications and few of them can be irreversible like neuropathy.⁵

Though high MCV is usually associated with B12 and folate deficiency but should not be solely relied upon, as it is not a significant and sensitive marker due to confounding factors like thalassaemia trait or iron deficiency.⁶ Concomitant deficiency of iron or presence of thalassaemia trait are very common in Pakistani population which would impair the identification of several cases of vitamin B12 or folate deficiency on the basis of macrocytosis only.² It might be possible that patients has marginally low levels of vitamin B12 deficiency without any clinical or lab
METHOD
Observational non-probability cross sectional study was conducted at DDRRL from January 2014 to September 2014. Approval was taken for ethical consideration from institutional review board of Dow University of Health Sciences (IRB-418/DUHS/-13). Sample size of 105 was calculated using open epi version 3.01, open source calculator with 7.35% prevalence of iron deficiency or thalassaemia trait in B12 deficient patients at 95% confidence interval. Cases referred from different OPD for lab investigation of vitamin B12 were selected according to inclusion criteria which were B12 level should be <200ng/L or 151 pmol/L and MCV < 95fl on CBC. Patients with a history of blood transfusion (3 months) and recent history of taking iron, B12 or folate supplements were excluded. After taking consent, questionnaire were filled by all the patients. Blood samples were collected for CBC, serum ferritin, RBC folate and Hb electrophoresis.

Anticoagulated whole blood samples for CBC were analysed on CELL DYN 3200 which utilises flow cytometric technique and peripheral smear were prepared within one hour of blood collection and stained with Leishman stain. Hb electrophoresis was performed to detect beta thalassaemia trait on Interlab Alkaline Hemoglobin Electrophoresis test system for qualitative and semi-quantitative determination. It was used for both normal and abnormal or variant hemoglobins using cellulose acetate strips at alkaline pH. The kit was used on Genios instrument. Serum ferritin was analysed on immulite 1000 analyzer by using a competitive chemiluminescent enzyme immunoassay. RBC folate was analysed on Cobas e 411 analyzer using Chemiluminescent Microparticle Immunoassay (CMIA).

RESULTS
Out of 105 vitamin B12 deficient cases, vitamin B12 deficiency was high in females with normal or low MCV i.e 62.85% (66) as compared to male 37.14% (39). Amongst 105 cases, 36.19%(38) were microcytic with the mean age of 37±16.2 years and 63.8% (67) were normocytic with the mean age of 41.58±16.5 years. Overall maximum number of cases were observed in age between 31 to 40 years followed by greater than 50 years. In microcytic group, the percent value of iron deficient, beta thalassaemia trait, combined deficiency of B12, iron and beta thalassaemia trait and RBC folate deficient were 52.6%,34.21%, 7.8% and 2.63% respectively. In normocytic group, the percent value of iron deficient, beta thalassaemia trait and RBC Folate deficient were 13.4%, 00% and 11.9% respectively.

During the study it was observed that 76.3%(29) and 29.85% (20) cases were anaemic with low and normal MCV respectively. Most of the anaemic cases 44.7% (17) with low MCV were due to combine B12 and iron deficiency while in group with normal MCV, most of the anaemic cases 14.% (10) were due to solo vitamin B12 deficiency. [Table-I-II]

None of vitamin B12 deficient cases had hypersegmented neutrophils or oval macrocytes on peripheral film while 30.4% (32) and 37.14%(39) cases had hypochromia and anisocytosis on morphology. There was only one case of pancytopenia (0.95%) and single case of bicytopenia (0.95%)

<table>
<thead>
<tr>
<th>No. of Cases (n/%)</th>
<th>Anaemic (20/29.85%)</th>
<th>Non-Anaemic (47/70%)</th>
<th>Pearson Chi-Square Test P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B12+ Iron deficient</td>
<td>8 (11.9%)</td>
<td>1 (1.49%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Vitamin B12 + Beta Thal Trait</td>
<td>00 (00%)</td>
<td>00 (00%)</td>
<td></td>
</tr>
<tr>
<td>Vitamin B12 + RBC Folate Deficient</td>
<td>2 (2.98%)</td>
<td>6 (8.95%)</td>
<td></td>
</tr>
<tr>
<td>Solo Vitamin B12 Deficient</td>
<td>10 (14.9%)</td>
<td>40 (59.7%)</td>
<td></td>
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</tbody>
</table>

Table-I. Anaemic vs non-anaemic distribution of vitamin b12 deficient cases presented with normal mcv (n=67)

*P < 0.05 was obtained by using Pearson Chi-Square Test
RBC: Red blood cell
MCV: Mean corpuscular volume
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DISCUSSION

Traditional criteria for measurement of vitamin B12 level is macrocytosis on peripheral blood smear and raised MCV on complete blood count.7,8 Though this is an important criteria but recent studies showed that B12 deficiency can also occur with normal or low MCV due to concomitant conditions that impairs the macrocytosis.9,10,11

The present study found 63.8% B12 deficient cases with normal MCV and 36.19% B12 deficient cases with low MCV. These findings are consistent with the findings of many studies.12,13,14 A study by Oosterhuis et al assessed the diagnostic accuracy of an elevated MCV for B12 deficiency and reported low sensitivity (17% to 30%) and that 84% of B12 deficient patients would have been missed if followed this criteria to diagnose B12 deficiency.15

Interestingly in our study B12 deficiency was seen more in younger age group as compared to other studies who reported B12 deficiency in elderly population.5,10 Furthermore, we found combined deficiencies more in females of reproductive age group and it could be due to increase demand, inadequate diet or loss of blood in regular menstrual cycles of these micronutrients in womans of reproductive age group. Therefore, B12 levels should be detected in all pregnant woman as it is well established fact that B12 level decreases physiologically during pregnancy and it may be the contributory cause of low birth weight, growth retardation or neural tube defects.16,17,18 In addition one of the study suggested that in pregnant woman B12 levels should be maintained greater than 300pg/ml.19 Current study found B12 deficiency with low or normal MCV in children including breast fed infant of a B12 deficient mother. The cause in infant might be due to maternal dietary deficiency which is generally apparent in breastfed infants at the age of 4 to 8 months.20

Current study affirmed 27.6% cases of simultaneous deficiencies of B12 and ferritin presenting with normal or low MCV. Our statistics also corresponds with the other studies.5,9,21,22 As B12 is required for DNA synthesis and its deficiency causes delay in maturation of erythroid progenitors in immature stages and iron deficiency causes decreased Hb synthesis

<table>
<thead>
<tr>
<th>No. of Cases (n/%)</th>
<th>Anaemic (29/76.3%)</th>
<th>Non-Anemic (9/23.6%)</th>
<th>Pearson Chi-Square Test P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B12 + Iron Deficient</td>
<td>17 (44.7%)</td>
<td>3 (7.89%)</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>Vitamin B12 + Beta Thal Trait</td>
<td>11 (28.9%)</td>
<td>02 (5.26%)</td>
<td></td>
</tr>
<tr>
<td>Vitamin B12 + RBC Folate Deficient</td>
<td>00 (00%)</td>
<td>04 (10.5%)</td>
<td></td>
</tr>
<tr>
<td>Solo Vitamin B12 Deficient</td>
<td>01 (2.63%)</td>
<td>00 (00%)</td>
<td></td>
</tr>
</tbody>
</table>

Table-II. Anaemic vs non-anaemic distribution of vitamin B12 deficient cases presented with low MCV (n=38)

*P < 0.002 was obtained by using Pearson Chi-Square Test

RBC: Red blood cell

MCV: Mean corpuscular volume
for a given time. Combined deficiencies of B12 and iron masked the morphological changes of B12 deficiency and treatment of iron deficiency anemia unrevealed the true B12 deficiency in these cases. Therefore, screening criteria of B12 deficiency based on MCV value might overlook a significant number of B12 deficient patients. Many studies suggested that in patients with Helicobacter pylori infections, significant low levels of both B12 and ferritin were found. Prevalence of H. Pylori infection in Pakistan is 74.4% which is quiet significant high. Significant body of literature showed the positive relation of H.Pylori infection and B12 deficiency. The pathophysiology of H.pylori infection in these micronutrients deficiency is still not clear.

We found significant number of cases of B12 deficiency with beta thalassaemia trait (12.3%). Many other studies also reported this combined deficiency. Estimated prevalence of thalassaemia carrier rate in Pakistan is around 5-8% with 9.8 million carrier in total population which is rising with time. Thus in thalassaemic patients who develop anaemia with or without neurological manifestations, further workup for megaloblastic pathogenesis should be done in the absence of macrocytosis.

Combined B12 and RBC folate deficiency was also observed in normocytic and microcytic group. We have not come across to any study reflecting the combined B12 and folate deficiency with low or normal MCV. We observed that significant number of cases of combine deficiencies showed anaemia with microcytic blood picture in comparision to B12 deficiency alone. We found similar findings by other studies.

In this research, 60% of cases with normal MCV were only B12 deficient. In these cases normal MCV might be due to coexistence of anaemia of chronic disorders as 35% of our B12 deficient cases with normal MCV gave history of chronic illnesses. The pathogenesis behind the anaemia of chronic disorders is release of cytokines and cells of reticuloendothelial cells which causes alteration in iron homeostasis, effects erythropoietin production and proliferation of erythroid progenitor cells. Similiar findings were also reported by one of the studies in China and India. Additional possibilities included other hemoglobinopathies like alpha thalassaemia trait as prevalence of alpha thalassaemia trait in Pakistan is approximately 2.4% which should be considered.

**CONCLUSION**

Many studies have been done in relation to vitamin B12 deficiency with high MCV but the data is scarce in this part of world relating to B12 deficiency with normal or low MCV. We suggest that physicians should include deficiency of B12 in their differential diagnosis while investigating anaemia irrespective of the value of MCV. Despite majority of our population was non vegetarian but probably malnutrition due to various causes restricted the accessibility and consumption of foods of animal origin.

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**REFERENCES**

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Stop wishing for it and start working for it.

– Unknown –