Evaluation of Vitamin B₁₂ and Folic Acid deficiencies in selective middle aged and elderly male and female patients

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ABSTRACT:
Significant prevalence of vitamin B₁₂ and folate deficiencies supported by biochemical evidence has been reported in the world. It was indicated that these biochemical evidences are associated with prevalence of anaemia in elderly. The major reasons of vitamin deficiencies, especially that of B₁₂, was reported to be inadequate dietary intake and, in the elderly, malabsorption of the vitamin from food. Vitamin deficiencies especially that of B₁₂ are usually diagnosed on the basis of serum or plasma vitamin concentrations. Due to dilemma of management and diagnoses of mal-nutrition and vitamin deficiencies in elderly population, the present study was undertaken to ascertain vitamin B₁₂, folate and RBC folate status in selected middle aged and elderly male and female patients. A total of 132 patients (period March 2004 to November 2007), were selected according to gender and age. For males (n = 72); age groups were 50-60, 61-70, 71-80 yrs and greater than 80 yrs and for females (n = 60); age groups were 52-61, 62-69, 70-79 and greater than 80 yrs. The results clearly depicts that elderly patients in both gender between the age groups of 71 and greater than 80 had significantly low vitamin concentrations (p < 0.001) than the middle age groups of 61 to 70 (p < 0.01). Correspondingly, their hemoglobin levels were also relates to the overall picture of either normal or low concentrations of vitamins in all groups. In males the lowest concentration of 3.5 ng/ml for folate, Vitamin B₁₂ of 228 pg/ml and 168 ng/ml for RBC folate were observed in > 80 years group preceded by 6.2 ng/ml, 278 pg/ml and 170 ng/ml respectively, in 71 to 80 years group, whereas in females, the observations were 2.5 ng/ml for folate, 220 pg/ml for B₁₂ and 110 ng/ml for RBC folate concentrations in > 80 years age group of patients. In conclusion few management strategies were suggested for therapy of vitamin deficient older patients.

Key words: Vitamin B₁₂, Folate, RBC folate, Deficiencies. Anemia

INTRODUCTION

Vitamin deficiencies especially that of B₁₂ are usually diagnosed on the basis of serum or plasma vitamin concentrations, with deficiency currently defined as a concentration lower than 200 pg/mL for B₁₂ and marginal deficient status defined as a concentration of 148–221 pmol/L. In 2005, a cutoff of 210 nmol/L has been proposed, ie, the 95th percentile for vitamin B₁₂-replete participants with normal renal function by National Health and Nutrition Examination Survey in the United States. The deficiency status of other vitamin parameters such as RBC folate and serum folate (Folic acid) are less than 176 ng/ml and 2.7 ng/ml respectively. Vitamin B₁₂ deficiencies are reported to be common in developed countries, particularly among the elderly, and are most prevalent in low income populations in under-developed countries around the world.

The evidence regarding reports that approximately one third of older adults or elderly have low folate, and/or vitamin B₁₂ deficiency relates to the fact that they are also anemic. In this regard, nearly two thirds being associated with iron deficiency due to chronic blood loss from gastrointestinal lesions. The remaining population or the patients that are attributed to vitamin deficiencies are usually associated with most frequently related to food-cobalamin malabsorption, and/or folate deficiency.

The prevalence of deficiency in USA population was reported by NHNE survey in 2005 (1,2,5) and shows that it affected nearly 3% of those aged 20–39 y, 4% of those aged 40–59 y, and 6% of persons aged nearly 70 yrs. Deficiency was present in 1% of children and adolescents but was nearly 3% in children aged 4 yrs. Similarly UK surveys shows the prevalence of vitamin B₁₂ deficiency (serum B₁₂ nearly 150 pmol/L)
increased substantially after age 69 yrs; it affected about 1 in 20 people aged 65–74 yrs and at least 1 in 10 of those aged > 75 yrs.

Because of the dilemma of management and diagnoses of mal-nutrition and vitamin deficiencies in elderly population, the present study was undertaken to ascertain vitamin B12, folate and RBC folate status in selected middle aged and elderly patients, both male and female to suggest appropriate diagnosis and management strategies of the same during old age, especially in the very elderly patients (> 80 years) to prevent adverse outcome.

MATERIALS AND METHODS
The study was carried out in 132 patients between the period March 2004 to November 2007, including the patients from both indoor and OPDs, and classified according to gender and age groups. For males (n=72); the age group were 50-60, 61-70, 71-80 yrs and greater than 80 yrs and for females (n=60); 52-61, 62-69, 70-79 and greater than 80 yrs. Serum folate, RBC folate and vitamin B12 measurements performed on a cohort of unselected subjects were referred by physicians and practitioners for routine diagnostic check-ups to our laboratory over the two years. Protocols of Lippi et al. was followed for processing and grouping of results. Blood samples from patients were routinely collected after an overnight fast. Haematological testing for Hemoglobin was performed on Nova Biomedical Phox analyzer (Nova Biomedical, USA). Serum folate, RBC folate and vitamin B12 were assayed on Elecsys 1010 and 2010 auto-immunoassay system (Roche Diagnostic, Basel). The total imprecision of the B 12 immunoassay is 3.7% CV and within precision 3.0%, for folate 5.0% CV and within precision 3.0% and RBC folate immunoassay is 5.5% CV and 3.5% within precision. Throughout the study the quality of results was validated by regular internal quality control procedures. Anaemia was defined as a haemoglobin concentration <14.0 g/dL in men and <12.3 g/dL in females, respectively. Low serum levels of folic acid, vitamin B12 and blood RBC folate were established at < 2.7 (normal 2.7-16.1 ng/ml; < 220 (220-925 pg/ml) and < 176 (176-590 ng/ml), respectively. The statistical significances of differences and frequency distribution of values were assessed by the Pearson’s correlation test and chi-squared test. The association between age and other variables was tested by multiple linear regression analysis. Statistical analyses were performed using the statistical package SPSS-version 13 (USA) and results are presented as the mean and standard error of the mean (SEM).

RESULTS:
Results were retrieved from the database of our indigenous visual basic-based Laboratory Information System for a total of 132 patients (period March 2004 to November 2007), both indoor and OPDs, according to gender and age groups which were for males (n=72); 50-60, 61-70, 71-80 yrs and greater than 80 yrs and for females (n=60); 52-61, 62-69, 70-79 and greater than 80 yrs (Table I). The number of male patients grouped in each age category were 28, 20, 17 and 7 respectively; whereas in female group; 20, 20, 14, and 6 respectively. The results clearly depicts that elderly patients in both gender between the age groups of 71 and greater than 80 had significantly low vitamin concentrations (p < 0.001) than the middle age groups of 61 to 70 (P < 0.01). Furthermore the middle age to aged group of 50 to 60 years have comparatively substantial concentration of B12, folate and RBC folate. Correspondingly, their hemoglobin levels were also relates to the overall picture of either normal or low concentrations of vitamins in all groups. The lowest concentration of 3.5 ng/ml for folate, Vitamin B12 of 228 pg/ml and 168 ng/ml for RBC folate were observed in > 80 years group preceded by 6.2 ng/ml, 278 pg/ml and 170 ng/ml respectively, in 71 to 80 years group. In females, the observations were nearly similar (but with more lower concentrations) with 2.5 ng/ml for folate, 220 pg/ml for B12 and 110 ng/ml for RBC folate concentrations in greater than 80 years age group of patients.

Percentage wise distribution of vitamin concentration were also cumulated and in males, 38.88% shows normal concentrations for all four parameters, 27.77% shows lower side of normal; whereas 23.66% and 9.6% which cumulatively depicts 33.33%, showed below normal or deficient concentration of vitamins and Hb. Similarly in female groups nearly 66.66% percent showed normal or near normal concentrations where as 33.33% (23.33% and 10%) were below normal or falls in vitamin deficient group.

DISCUSSION:
Significant prevalence of vitamin B12 and folate deficiencies supported by biochemical evidence has
been reported in the world\textsuperscript{1,3}. It was indicated that these biochemical evidences are associated with prevalence of anaemia in elderly\textsuperscript{8}. However, several data suggest otherwise\textsuperscript{3}. The major reasons of vitamin deficiencies, especially that of B-12, was reported to be inadequate dietary intake and, in the elderly, malabsorption of the vitamin from food\textsuperscript{1}. Interestingly, strict vegetarians (vegans) are also at high risk of vitamin B-12 deficiency\textsuperscript{1,3}. Estimated Average requirement in USA and Canada is 0.7–2.0 μg/d across the life span, whereas the respective recommended dietary allowance is 0.9–2.4 μg/d. Vitamin B-12 is present only in animal-source foods (ASFs) or fortified foods and therefore in a large study carried out in United Kingdom, depicts that vitamin intakes increased progressively with ASF intake, averaging 0.4 μg for vegans, 2.6 μg for lactovo-vegetarians, 5.0 μg for those who also consumed fish, and 7.2 μg for consumers of meat (omnivores)\textsuperscript{1,9}. Numerous other studies on smaller population groups confirmed that both vitamin B-12 intake and serum vitamin B-12 concentrations increase progressively from vegans to lactoovo-vegetarians, to those who consume fish or some meat, to omnivores\textsuperscript{10,11}. A study carried out by Loikas et al\textsuperscript{11} regarding vitamin B-12 deficiency and includes previously diagnosed in 27 (2.6%) subjects, and a laboratory diagnosis (total vitamin B-12 <150 pmol/l, or total vitamin B-12 150-250 pmol/l and holotranscobalamin < or =37 pmol/l and homocysteine > or =15 micromol/l) was made for 97 (9.5%) subjects. It is also reported that low serum total vitamin B12 (<150 pmol/l) was observed in 6.1% and borderline total vitamin B12 (150-250 pmol/l) in 32% of the subjects. Those male gender (OR 1.9, 95% CI 1.2-2.9), age > or =75 (OR 2.2, 95% CI 1.4-3.4) who doesn't consume milk products (OR 2.3, 95% CI 1.2-4.4) have the probability for vitamin B12 deficiency. It was concluded by the study that undiagnosed vitamin B12 deficiency is remarkably common in the aged, but no specific risk group for screening can be identified.

Previously it was well documented that Vitamin B12 deficiency occurs frequently among elderly patients\textsuperscript{12,13}. Furthermore, it is often unrecognized or not investigated because the clinical manifestations are subtle\textsuperscript{13}. However, the potential seriousness of the complications (particularly neuropsychiatric and hematological)\textsuperscript{12,14,16} requires investigation of all patients who present with vitamin or nutritional deficiency\textsuperscript{13}. Epidemiological studies show a prevalence of B 12 deficiency of around 20% (between 5% and 60%, depending on the definition of cobalamin deficiency used in the study) in the general population of developed countries\textsuperscript{13}. A study carried out in Framingham demonstrated a prevalence of 12% among elderly people living in the community\textsuperscript{17}. Other studies focusing on elderly people, particularly those who are under medical care in hospitals and who are sick, have suggested a higher prevalence: 30% - 40%\textsuperscript{13,18,19}. Andres et al\textsuperscript{13} in 2004 found that the prevalence of greater than 4.8% exist in a large group of patients in hospital between the ages of 65 and 98 (data submitted to the 47th Congress of the French National Society for Internal Medicine in Bordeaux, June 11–13, 1998).

In elderly patients, cobalamin deficiency is caused primarily by food-B12 malabsorption and pernicious anemia. B12 or more commonly known as cobalamin deficiency caused by diet deficiency or malabsorption is rarer\textsuperscript{12,13,18,20}. In several studies carried out over the years, in which more than 200 patients were examined with a proven cobalamin deficiency, food- B12 malabsorption accounted for about 60%–70% of the cases among elderly patients, and pernicious anemia accounted for 15%–20% of the cases\textsuperscript{13,21,24}. Other causes included dietary deficiency (<5%), malabsorption (<5%) and hereditary cobalamin metabolism diseases (< 1%).

Normally, humans maintain a large vitamin B12 reserve, which can last two to five years even in the presence of severe malabsorption\textsuperscript{25}. Nevertheless, nutritional deficiency can occur in specific populations. Elderly patients with “tea and toast” diets and chronic alcoholics are at especially high risk. The dietary limitations of strict vegans make them another, less common at-risk population\textsuperscript{26}. The classic disorder of malabsorption is pernicious anemia, an autoimmune disease that affects the gastric parietal cells. Destruction of these cells curtails the production of intrinsic factor and subsequently limits vitamin B12 absorption\textsuperscript{26}. In conclusion, certain measures and therapeutic regiments have been suggested as reported by several studies. Andres et al\textsuperscript{13} suggests that classic treatment in deficiency, particularly when the cause is not dietary deficiency, has been parenteral administration — usually by intramuscular injection — of the vitamin (in the form of cyanocobalamin and, more rarely,
hydroxocobalamin). Another study in France, recommended practice to build up the tissue stores of the vitamin quickly and correct serum cobalamin hypovitaminosis, particularly in the case of pernicious anemia, involves administration of 1000 μg of cobalamin per day for 1 week, followed by 1000 μg per week for 1 month and then by 1 injection of the same dose once per month, normally for the rest of the patient's life. In other Western countries, dosages of 100–1000 μg per day are used.

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Table I A: Folic acid, Vitamin B 12 and RBC folate concentrations in males (n = 72) of middle aged and elderly groups.

<table>
<thead>
<tr>
<th>Age groups</th>
<th>50-60 yrs</th>
<th>61-70 yrs</th>
<th>71-80 yrs</th>
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<tr>
<td>Numbers</td>
<td>28 (38.8%)</td>
<td>20 (27.77%)</td>
<td>17 (23.61%)</td>
<td>7 (9.70%)</td>
</tr>
<tr>
<td>Hb g/dL</td>
<td>14.3 ± 2.3</td>
<td>14.0 ± 2.1</td>
<td>13.4 ± 1.4</td>
<td>13.1 ± 1.1</td>
</tr>
<tr>
<td>Folate (ng/ml)</td>
<td>13.4 ± 3.2***</td>
<td>10.2 ± 1.5</td>
<td>6.2 ± 0.35**</td>
<td>3.5 ± 0.45***</td>
</tr>
<tr>
<td>B 12 (pg/ml)</td>
<td>460.5 ± 41.2***</td>
<td>401.8 ± 32.2**</td>
<td>278.6 ± 18.9 ***</td>
<td>228.5 ± 22.4***</td>
</tr>
<tr>
<td>RBC folate (ng/ml)</td>
<td>219.7 ± 25.6**</td>
<td>198.7 ± 17.6*</td>
<td>170.4 ± 24.1</td>
<td>168.6 ± 20.6*</td>
</tr>
</tbody>
</table>

Normal population references ranges: Folate = 2.7-16.1 ng/ml; B 12 = 220-925 pg/ml; RBC folate = 176-590 ng/ml.

* significantly differ (< P 0.05) with the corresponding groups
** significantly differ (< P 0.01) with the corresponding groups
*** significantly differ (P < 0.001) with the corresponding groups

Table I B: Folic acid, Vitamin B 12 and RBC folate concentrations in females (n = 60) of middle aged and elderly groups.

<table>
<thead>
<tr>
<th>Age groups</th>
<th>52-61 yrs</th>
<th>62-69 yrs</th>
<th>70-79 yrs</th>
<th>&gt; 80 yrs</th>
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<tr>
<td>Numbers</td>
<td>20 (33.33%)</td>
<td>20 (33.33%)</td>
<td>14 (23.33%)</td>
<td>6 (10%)</td>
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<tr>
<td>Hb g/dL</td>
<td>12.3 ± 3.2</td>
<td>12.0 ± 2.9</td>
<td>11.01 ± 2.2</td>
<td>11.10 ± 1.3</td>
</tr>
<tr>
<td>Folate (ng/ml)</td>
<td>13.0 ± 2.0***</td>
<td>9.1 ± 2.1</td>
<td>5.2 ± 0.87**</td>
<td>2.5 ± 0.43***</td>
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<tr>
<td>B 12 (pg/ml)</td>
<td>410.5 ± 45.5***</td>
<td>388.6 ± 33.1**</td>
<td>240.4 ± 26.7***</td>
<td>220.7 ± 23.5***</td>
</tr>
<tr>
<td>RBC folate (ng/ml)</td>
<td>210.4 ± 22.3**</td>
<td>178.6 ± 15.2*</td>
<td>148.6 ± 16.3**</td>
<td>110.3 ± 14.6**</td>
</tr>
</tbody>
</table>

Normal population reference ranges: Folic acid = 2.7-16.1 ng/ml; B 12 = 220-925 pg/ml; RBC folate = 176-590 ng/ml.

* significantly differ (< P 0.05) with the corresponding groups
** significantly differ (< P 0.01) with the corresponding groups
*** significantly differ (P < 0.001) with the corresponding groups