

# Evaluation of Anaemia in Type 2 Diabetes Mellitus Patients with Special Reference to Vitamin B12 Deficiency

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**Abstract:** ***Background:** Anemia is prevalent in patients with diabetes. It predicts the progression of diabetic complications. <sup>1</sup>Vitamin B12 deficiency is also a potential comorbidity in diabetes mellitus. <sup>2</sup>The aim of this study was to evaluate anaemia in patients with type 2 diabetes mellitus with normal renal function with special reference to vitamin B12 deficiency. **Methods:** This study was an observational study conducted where 190 cases > 13 years of age with type 2 diabetes mellitus having eGFR >60 ml/min; serum creatinine < 1.5 mg/dl; urine albumin to creatinine ratio < 300 mg/g were selected. Anaemia was defined using World Health Organization criteria: haemoglobin <13 g/dl for men, <12 g/dl for women. Vitamin B12 estimation was done by the principle of ELISA. The statistical significance was fixed at 5% level (p value <0.05). **Results:** The prevalence of anaemia in type 2 diabetics with normal renal function was 36.32% with male to female ratio 0.7: 1 and mean age 56.11±18.75 years with major type being normocytic. Duration of diabetes, daily metformin dose >1000mg, duration of metformin use >5 years, hypertension, dyslipidemia, poor glycemic control, microalbuminuria were found to be statistically significant risk factors. The prevalence of diabetic complications was higher in patients with anaemia. The prevalence of Vitamin B12 deficiency was 17.36% being an independent risk factor for anaemia. (p=0.113; OR=1.9). **Conclusion:** Anaemia is prevalent in type 2 diabetics even before the onset of renal impairment with higher prevalence of complications (microvascular mainly studied here) which increased with increasing severity of anaemia. Vitamin B12 deficiency is prevalent in diabetics with anaemia.*

**Keywords:** Type 2 Diabetes Mellitus, Anaemia, Vitamin B12 deficiency

## 1. Introduction

Diabetes mellitus is a disease of metabolic dysregulation characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism. Anemia is commonly found in patients with diabetes. It causes reduced exercise capacity, fatigue, anorexia, depression, cognitive dysfunction, decreased libido, increases cardiovascular risk and also predicts the progression of micro- and macrovascular complications. Though anemia in diabetics is mostly associated with the presence of renal insufficiency, there is also a high prevalence before the onset of renal impairment. <sup>1</sup> Similarly, Vitamin B12 deficiency is a potential comorbidity in diabetes mellitus that is often overlooked, despite many diabetic patients being at an increased risk. Many diabetic patients are treated with metformin, a medication that lowers serum vitamin B12 levels. In addition, almost half of all diabetic patients are older than 60, an age group in which the prevalence of metabolically confirmed B12 deficiency is high. Because of these risk factors, defining the prevalence of vitamin B12 deficiency in the diabetic population may help determine whether primary care physicians should consider screening for B12 deficiency in diabetic patients. <sup>3</sup> It is thus obvious that there is an absolute necessity for early detection of anaemia (before the onset of renal impairment) and vitamin B12 deficiency in diabetics in order to help prevent and decrease the progression of complications of diabetes as well as to improve the quality of life. Hence this study attempts to evaluate anaemia in patients with type 2 diabetes mellitus patients without renal insufficiency with special reference to vitamin B12 deficiency.

## 2. Methods

This was an observational study conducted during the period of one year from June 2019 to May 2020 which was carried out after obtaining approval from Institutional Ethics Committee. A total of 190 cases with age more than or equal to 13 years admitted in Department of Medicine, Assam Medical College and Hospital, Dibrugarh who were diagnosed indoor and outdoor cases of type 2 diabetes mellitus with normal renal function (serum creatinine <1.5mg/dl and eGFR > 60ml/min. Proper medical history specifically with respect to symptoms of anaemia and vitamin B12 deficiency were elicited. General and systemic physical examination was done. Routine examination of Blood, peripheral blood smear, iron supply studies, Renal Function Tests, FBS, PPBS, HbA1c levels, Fasting lipid profile, Liver Function Test, Serum electrolytes, Routine examination of urine, urine for microalbuminuria, 24-hour urinary protein, stool examination for occult blood, routine and microscopy for ova and cyst, Electrocardiogram, Echocardiography, Ultrasound whole abdomen, bone marrow examination and upper GI endoscopy were done if indicated. Assessment of coexisting retinopathy was done using HEINE mini 3000 direct ophthalmoscope. Assessment of diabetic peripheral neuropathy was done using a 10 g Semmes Weinstein monofilament and nerve conduction study using Medicaid EMG 2000 NEURO PERFECT EMG/NCS/EP system. Diabetic foot examination was done by clinical examination. Vitamin B12 estimation was done by the principle of ELISA using two AccuDiag Vitamin B12 ELISA kits. Definite Vitamin B12 deficiency was defined as serum levels of B12 < 150 pg/ml and possible deficiency as 150-220 pg/ml and values > 220 pg/ml were taken to be

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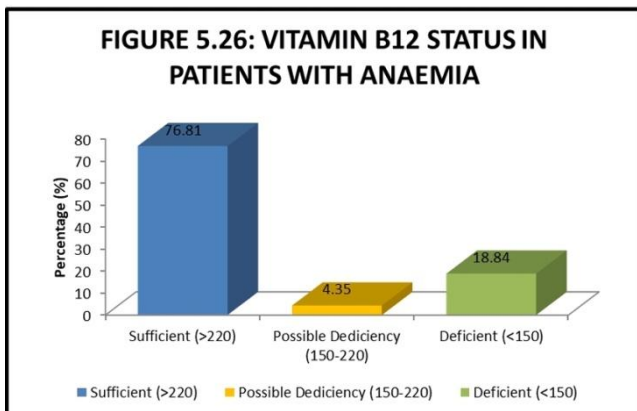
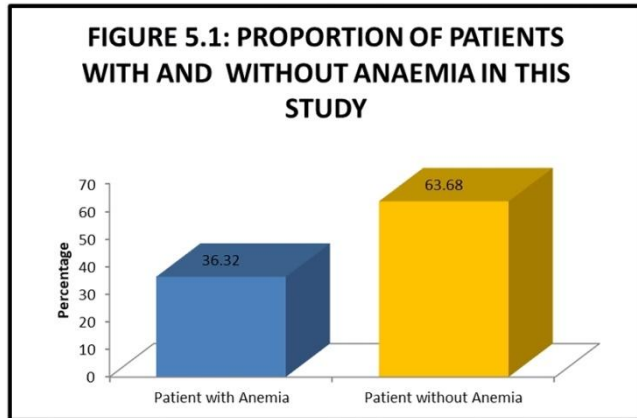
sufficient. For all analyses, the statistical significance was fixed at 5% level ( $p$  value  $<0.05$ ).

### 3. Results

Out of 190 known cases of type 2 diabetes 113 (59.4 %) were males and remaining 77 (40.53%) patients were females. Among them 69 (36.32%) of the patients were found to have anaemia. The ratio of male to female in patients with anaemia group is 0.7: 1 while the ratio in non-anaemia group is 2.4: 1. Majority were found to belong to 41-60 years of age. Patients with anaemia were older but not significantly so ( $p=0.14$ ). Among 190 cases, 121 (63.68%) patients were found to have hemoglobin in the normal range ( $>12.5$ gm/dl), 29 patients (15.26%) had moderate anaemia in the range of 7.1-9.9gm/dl, 26 (13.68%) patients had mild anaemia in the range of 10-12.4gm/dl and 14 (7.37%) patients had severe anaemia (4.1-7gm/dl). None of the patients had very severe anaemia ( $< 4$ gm/dl). Out of 69 patients with anaemia majority were found to have moderate and mild anaemia. 14 (20.3%) patients had severe anaemia while none of them had very severe anaemia. Microcytic hypochromic anaemia constituted the major percentage (39.13%), followed by normocytic normochromic (34.78%), normocytic hypochromic (17.39%), macrocytic (7.25%), dimorphic (1.45%). The mean duration of diabetes mellitus in patients with anaemia was found to be  $8.01\pm 6.35$  years and in those without anaemia was  $5.35\pm 3.82$  years. ( $p=0.02$ ). Duration of diabetes  $>5$  years was a significant risk factor for development of anaemia in diabetics ( $p=0.02$ , OR=2.05). Alcohol usage was found to be a significant contributor to development of anaemia in diabetics ( $p=0.0005$  OR=1.33). The association between development of anaemia and smoking was not found to be statistically significant. The use of ACEi/ARBs was not found to be a significant risk factor to the development of anaemia in patients with type 2 diabetes mellitus. ( $p=0.9$ , OR=0.96). The patients with anaemia had a higher daily dose of metformin with a mean of  $1125.81\pm 641.64$  mg/day as compared to a mean of  $856.68\pm 476.52$  mg/day in patients without anaemia ( $p=0.0012$ ). Thus daily dosage of metformin  $>1000$ mg/day was identified to be a risk factor to development of anaemia in type 2 diabetics. ( $p=0.009$ , OR=2.25). The mean duration of metformin use in patients with anaemia was higher ( $6.46\pm 5.25$  years) as compared to  $4.12\pm 3.45$  years in patients without anaemia. ( $p=0.0003$ ). The duration of metformin use of  $>5$  years was found to be a significant risk factor in development of anaemia. ( $p=0.005$ , OR=2.36). Higher percentage (71.02%) of patients with anaemia were found to use insulin as compared to 34.71% of patients without anaemia. Use of insulin was found to be a significant risk factor for development of anaemia ( $p<0.0001$ , OR=4.6). The mean systolic blood pressure of patients with anaemia was higher ( $137.33\pm 29.17$ mm of Hg) than that of patients without anaemia ( $127.59\pm 18.63$  mm of Hg) [ $p=0.0044$ ]. The mean diastolic blood pressure in patients with anaemia was higher ( $82.04\pm 13.29$  mm of Hg) than in those without anaemia ( $76.36\pm 11.64$  mm of Hg) [ $p=0.0025$ ]. Hypertension was found to be a risk factor for development of anaemia. The mean fasting blood sugar of patients with anaemia was found to be higher ( $136.21\pm 57.68$ mg/dl) as compared to  $127.81\pm 39.25$  mg/dl in patients without anaemia. High fasting blood sugar was

found to be a risk factor. ( $p=0.03$ ). Glycemic control was found to be significantly poor in patients with anaemia and was identified as a significant risk factor for development of anaemia in diabetics. Mean HbA1c in patients with anaemia was  $9.66\pm 2.78\%$  ( $p=0.0003$ ). Dyslipidemia was identified as a statistically significant risk factor. Higher mean total cholesterol levels of  $179.2\pm 29.0$  g/dl ( $p<0.001$ ), higher mean LDL of  $122.09\pm 39.43$  ( $p=0.024$ ), lower mean HDL levels of  $31.26\pm 8.41$  (0.0001) were found in patients with anaemia. Higher percentage (63.76%) of patients with anaemia had microalbuminuria as compared to 42.97% of patients without anaemia. Microalbuminuria was found to be a significant risk factor ( $p=0.0063$ , OR=2.33). 4 patients of anaemia with duration of diabetes  $<1$  month duration have mild anaemia. Majority (81.81%) of cases with diabetes of duration of 1 month to 1 year had mild anaemia. Majority (59.09%) of cases with duration of diabetes 6 to 10 years and 50% of cases with duration of 11 to 15 years had moderate anaemia. 100% and 75% cases with HbA1c of 5.6-7% and 7.1-8.4% respectively had mild anaemia. Majority of cases with HbA1c of 8.5-12.9% had moderate anaemia. 60% of cases with HbA1c $>13\%$  had severe anaemia. This shows an increasing severity of anaemia with worsening glycemic control. Majority of cases with daily dosage of metformin of 1500 and 2000 mg had moderate to severe anaemia. In patients who have taken metformin for  $>10$  years majority of cases (54.54%) had severe anaemia. Among patients with retinopathy 56.41% of patients had anaemia. Among patients with peripheral neuropathy 52.17% had anaemia. Major percentage of patients with anaemia (63.77%) had microalbuminuria as compared to 42.98% of patients without anaemia had microalbuminuria. Among patients with anaemia, 24.64% of patients had diabetic foot complications. It has been found that prevalence of complications was higher in patients with anaemia as compared to those without anaemia. Majority (53.62%) of patients had MCV in the normal range of 80-95. out of total 69 cases major percentage-Majority (63.77%) of the cases had serum ferritin in the normal range of 20-200ng/ml. Major percentage of patients (75.36%) had normal serum iron levels in the range of 60-170 mcg/dl. Majority of the patients had serum TIBC in the low to normal range. There was a statistically significant difference in vitamin B12 levels of patients with and without anaemia. The diabetic patients with anaemia had lower mean vitamin B12 level of  $450.29\pm 264.06$  ( $p=0.0011$ ). In this study prevalence of Vitamin B12 deficiency was found to be 17.36% (33 out of 190 cases). Out of them 15 (7.89%) had possible deficiency with levels between 150-220 ng/ml and 18 (9.47%) had levels less than 150 ng/ml. out of 69 patients with anaemia 76.81% were Vitamin B12 sufficient, 4.35% of patients had possible deficiency and 18.84% of patients were vitamin b12 deficient. Male gender, daily metformin dose of  $>1000$ mg, chronic PPI use, may be contributing factors to development of vitamin b12 deficiency. But statistical significance of these factors could not be established due to small sample size and confounding factors. Coexisting microalbuminuria, neuropathy and retinopathy are not associated with increased risk of vitamin b12 deficiency (OR $<1$ ). Use of metformin and sulfonylurea combination drugs was found to be a significant risk factor for vitamin B12 deficiency. ( $p=0.05$ , OR=4.59). No relation

was found between vitamin b12 levels and severity of anaemia.



#### 4. Discussion

Out of 190 patients included, 36.3% of patients were found to have anaemia. In a study conducted by Adiv-Goldhaber et al,<sup>4</sup> prevalence of anaemia among patients with diabetes with normal renal function was found to be 32%. In another study by Jessica Barbieri et al,<sup>5</sup> found that prevalence of anaemia in diabetics with renal insufficiency was found to be 34.2%. Hence diabetic females had a higher rate of anaemia as compared to their male counterparts. This is comparable to a study done by Faisal Alsayegh et al,<sup>6</sup> in which male: female ratio was found to be 0.8: 1. The patients with anaemia were found to be older with mean age being 56.11±18.75 years as compared to those without anaemia 52.99±10.98 years but not statistically significant (p=0.14) In several studies done by Faisal Alsayegh et al,<sup>6</sup> Babatunde Ishola Adejumo,<sup>7</sup> Catherine XR chen et al<sup>8</sup>; it was found that there was greater risk of anaemia in older patients as compared to younger age groups. Majority (52.17%) was found to be normocytic anaemia, out of which 24 cases (34.78%) were normocytic normochromic anaemia and 12 cases were normocytic hypochromic. In a study by Afreen sajid et al,<sup>9</sup> most prevalent type of anaemia was found to be normocytic normochromic anaemia. This finding can be attributed to the fact that diabetes mellitus is a state of chronic inflammation causing suppression and apoptosis of erythroid progenitor cells leading to erythropoietin deficiency and hyporesponsiveness to erythropoietin which is not only found in renal insufficiency but has also been found to occur even before the onset of renal impairment in diabetics. Nutritional deficiencies such as iron, vitamin B12, folate

deficiencies might also contribute. This is consistent with the fact that anaemia in diabetes is multifactorial.<sup>10</sup> In this study 63.77% of patients with anaemia had serum ferritin in the normal range. This is comparable to a study by Yong seok yun et al,<sup>10</sup> in which mean serum ferritin levels were found to be 198.5±122.9. Majority of the patients also had normal serum iron and TIBC in the low to normal range. This is consistent with the findings of case control study by Samuel Antwi Baufor et al.<sup>11</sup> Hence it can be suggested that the major cause of anaemia in our study may be due to anaemia of chronic disease rather than iron deficiency. Daily dosage of metformin >1000mg/day was identified to be a risk factor to development of anaemia in type 2 diabetics. (p=0.009, OR=2.25). The duration of metformin use of >5 years was also found to be a significant risk factor in development of anaemia. (p=0.005, OR=2.36). In a study by Louise A. Donneley et al,<sup>12</sup> it was shown for the first time that metformin use is associated with the risk of anemia in individuals with type 2 diabetes. Metformin impairs absorption of vitamin B12, and serum vitamin B12 levels are inversely correlated to dose and duration of metformin therapy Hypertension was found to be a risk factor for development of anaemia [p=0.0044], [p=0.0025]. Studies done by Shokoufeh Bonakadaran et al, (p=0.001) and Catherine XR Chen et al, (p<0.001) also showed significant association between hypertension and anaemia. This association can be attributed to the fact that hypertension is also a state of chronic inflammation. High fasting blood sugar was found to be a risk factor. (p=0.03) In a study by Samuel Antwi-Bafour et al., (p<0.0001) and Babatunde Ishola Adejumo et al, (p<0.01), showed significant association between high fasting blood glucose and anaemia. This could be attributed to the reason that poorly controlled diabetes can lead to anaemia. In this study glycemic control was found to be significantly poor in patients with anaemia and was identified as a significant risk factor for development of anaemia in diabetics. This shows an increasing severity of anaemia with worsening glycemic control similar to studies by Samuel Antwi-Bafour et al. (p<0.05), Babatunde Ishola Adejumo et al. (p=0.01), Debashish paul et al., (p<0.0001), Salma M. Aldallal et al., (p<0.05). In this study dyslipidemia was identified as a statistically significant risk factor (p<0.001). In a study by Bin-Bin He et al,<sup>13</sup> showed significant association between dyslipidemia and anaemia (p<0.001). In this study it was found that the patients with anaemia had higher odds of having diabetic retinopathy as compared to those without anaemia (p=0.042, OR=2.86). In his study Shokoufeh Bonakadaran et al., 2011 found that retinopathy was more frequent in diabetic patients with anaemia than those without anaemia. (p=0.001). In a study by Bin Bin He et al., 2015 found that anaemia was independently associated with diabetic retinopathy. In studies by G. Deray et al., Chagai grossman et al. and Faisal Alsayegh et al. also showed that patients with anaemia had an increasing risk of retinopathy and often exhibit severe lesions. Also showed that severity of anaemia increased with severity of retinopathy. In our study it was found that higher percentage (63.76%) of patients with anaemia had microalbuminuria as compared to 42.97% of patients without anaemia. Microalbuminuria was found to be a significant risk factor (p=0.0063, OR=2.33). G. Deray et al, showed that patients with persistent microalbuminuria had 4 times and patients with



macroalbuminuria 12 times the risk of anemia compared to normoalbuminuric subjects. Bin Bin He et al, showed significant association between microalbuminuria and anaemia ( $p=0.001$ ). In this study it was found that among patients with peripheral neuropathy 52.17% had anaemia which was higher as compared to those without anaemia (18.18%). [ $p=0.0001$ ;  $OR=3.6$ ] It was also found that among patients with anaemia, 24.64% of patients had diabetic foot complications as compared to 11.57% in patients without anaemia ( $p=0.021$ ;  $OR=2.49$ ). In a study by Bin bin he et al, the prevalence of anemia was much higher in patients with DPN than in those without DPN, and logistic regression showed that anemia was a risk factor of DPN after adjusting for conventional diabetes-related risk factors. In another study by Faisal Alsayegh et al, found that diabetic patients with anemia had higher presence of peripheral neuropathy and diabetic foot ( $p < 0.001$ ). In this study the prevalence of vitamin B12 deficiency was found to be 17.36. The presence of vitamin b12 deficiency was found to be an independent risk factor for development of anaemia in our study. ( $p=0.113$ ;  $OR=1.9$ ). A study conducted by Pflipsen MC et al, <sup>14</sup>found 22% type 2 diabetic patients to have metabolically confirmed vitamin B12 deficiency which is comparable to our study. Vitamin B12 deficiency is one of the most clinically important side effects of metformin therapy. Approximately 6-10% and upto 30% of patients receiving metformin may have reduced vitamin B12 absorption. Wile et al, Tomkin et al, and Defronzo et al, reported that serum vitamin B12 levels were found to be decreased in 14-30% of patients treated with metformin. In this study there was no significant difference between hemoglobin levels of vitamin B12 sufficient and deficient groups. ( $p=0.13$ ). Also there is no statistically significant difference in the RBC indices of the deficient and sufficient groups. This is consistent with the finding of a study done by Donghoon Kang et al. This may be due to reason that the cause of anaemia was most likely due to chronic disease which was established by this study in previous observations.

## 5. Conclusion

Thus we found that anaemia is a prevalent finding in patients with diabetes mellitus even before the onset of renal impairment. Duration of diabetes, usage of alcohol, daily metformin dose >1000mg, duration of metformin, duration of metformin use >5 years, insulin use, hypertension, dyslipidemia, high fasting blood sugars, poor glycemic control, microalbuminuria were risk factors associated with incidence of anaemia in type 2 diabetics with normal renal function. It was also found that diabetic patients with anaemia have a higher prevalence of complications (microvascular mainly studied here) which increased with increasing severity of anaemia. It became clear that vitamin B12 deficiency is a potential comorbidity in type 2 diabetic patients. Although vitamin B12 deficiency was found to be risk factor it cannot be attributed as the sole cause of anaemia in type 2 diabetics. Other potential causes of anaemia also should be kept in mind. High doses of metformin for prolonged duration, sulfonylurea and metformin combination drugs were significantly associated with vitamin B12 deficiency. Moreover, anaemia predicts progression of diabetic complications. . It has been shown to

be an independent risk factor for reduced cardiac and renal function as well as being associated with exacerbations of congestive heart failure in patients with heart disease. Correction of anemia may have a significant role in prevention of other diabetic complications. Thus we recommend that treatment criteria for diabetes should include routine hematological tests and take into consideration the inevitable consequences of aging, and poor glycemic control, in order to make optimal therapeutic decisions for the treatment of diabetes mellitus in adults. We also suggest the need for regular vitamin B12 monitoring in patients with type 2 diabetes, particularly patients receiving higher daily dosage of sulfonylurea plus metformin treatment for a long time period, even in the absence of hematological abnormalities.

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