

## Original Article

# An Observational, Cross-Sectional Study on Haematological Abnormalities in Patients with Type 2 Diabetes Mellitus on Metformin for 5 Years or More in a Tertiary Care Center

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## ABSTRACT

**Introduction:** Metformin, the preferred first line oral hypoglycaemic agent used in type 2 diabetes mellitus, is notorious to cause vitamin B12 deficiency which can lead to reversible macrocytic anaemia and/or neurological dysfunction. A simple and inexpensive test like complete blood count could go a long way in timely diagnosis and treatment of anaemia.

**Aims and objectives:** To observe the haematological abnormalities in patients using Metformin for 5years or more and to note the effects of age, sex, residence of the subjects, drug dose, duration and combination with other oral hypoglycaemic agents on such changes.

**Methodology:** In this observational and cross-sectional study, 100 patients aged 18-60 years with type 2 Diabetes Mellitus using Metformin for 5years or more were recruited after exclusion of use of drugs (vitamin or iron supplements, antiplatelets etc.) and alcohol, hypothyroidism, end-organ disease. They were subjected to complete blood count with RBC indices. Serum iron profile was sent for the microcytic group and serum vitamin B12 and folate estimation were done in the macrocytic group.

**Results:** 66 out of 100 were having anaemia of following types- 44 normocytic normochromic, 19 microcytic hypochromic (84.2% are iron-deficient) and 3 macrocytic (66.7% B12 deficient). Anaemia is more frequent in females aged 18-50y (71.8%), rural individuals (76.6%), people using increased daily dosage or insulin in combination with Metformin ( $p < 0.05$ ). Nevertheless no such relation could be elicited between duration of Metformin use and anaemia nor does Metformin show any effect on leukocyte or platelet counts.

**Conclusion:** Regular screening is imperative by physicians in such cases for timely detection of different types of anaemia and prompt treatment.

**Keywords:** Metformin, Type 2 Diabetes Mellitus, Haematological Changes, Anaemia

## INTRODUCTION

Diabetes mellitus is rapidly gaining the status of a potential epidemic and is predicted to afflict up to 79.4 million people in India by 2030. Biguanides were first clinically developed in 1950s by French physician Jean Sterne. Metformin, the prototype, is deemed as the first-choice therapy for type 2 diabetes mellitus (T2DM) due to its efficacy in decreasing insulin resistance and cardiovascular risk.<sup>1,2,3,4</sup>

It curbs hepatic glucose output, primarily by inhibition of gluconeogenesis, stimulates fatty acid oxidation, increases glucose uptake by skeletal muscles and

insulin sensitivity by increasing the activity of the Adenosine monophosphate-dependent protein kinase (AMPK) besides having antineoplastic and neuroprotective effects.<sup>5</sup> Apart from side effects like gastric intolerance and dizziness, studies have shown that the prevalence of Metformin-induced vitamin B12 deficiency ranged between 5.8 % and 52 % which can lead to reversible macrocytic anaemia and/or neurological dysfunction. Changes in small bowel motility, alterations in the bacterial flora, competitive inhibition, the effect of calcium on cell membranes have been suggested to cause B12 malabsorption.<sup>6</sup>

Anaemia is quite common problem in this part of the country and can be attributed to different etiologies. There are few studies which explored them or analysed prolonged Metformin use as a possible cause for anaemia. The present study stems from the fact that anaemia, often neglected, has high morbidity and mortality that can be easily reversed if detected early by simple and cheap blood counts.

### Aims and objectives

The objectives of the present study is to observe the haematological abnormalities in patients using Metformin for 5years or more and to note the effects of age, sex, residence of the subjects, drug dose, duration and combination with other oral hypoglycaemic agents on such changes.

### METHODOLOGY

A total of 100 patients aged 18-60 years with type 2 Diabetes Mellitus using Metformin for 5years or more who were admitted as in-patients or under outpatient department follow up of department of General Medicine and Endocrinology, Medical College & Hospital, Kolkata from January 2017 to June 2018 were included in our study. Written informed consent was obtained from the patients and the study protocol was approved by Institutional Ethics Committee for human research, Medical College, Kolkata. Diabetes was diagnosed according to latest ADA guidelines i.e. fasting plasma glucose  $\geq$  126 mg/dl, 2hour post prandial/random  $\geq$  200mg/dl and/or HbA1C  $\geq$  6.5%. People who were vegetarian, hypothyroid, alcoholics, on vitamin or iron supplements, antiplatelets, methotrexate, H2 receptor blocker or proton pump inhibitors, suffering from chronic kidney or liver disorder or haematological disorder were excluded. The present study was an observational, cross-sectional, analytical and single centre based study without any timed follow up.

After history taking, review of past records and clinical examination, all the participants were subjected to complete blood count with RBC indices (by automated cell counter and peripheral smear examination). The World Health Organization (WHO) defines anaemia as a haemoglobin level  $<$ 130 g/L (13 g/dL) in men and  $<$ 120 g/L (12 g/dL) in women. For diagnostic purpose mean corpuscular volume (MCV) is taken to be less than 80fL and mean corpuscular haemoglobin (MCH) is taken to be less than 27 pg/cell so as to consider microcytic hypochromic anaemia and mean corpuscular volume (MCV) is taken to be more than 100fL for diagnosis of macrocytic anemia.<sup>7</sup>

Serum iron profile was sent for the microcytic group and serum vitamin B12 and folate estimation (in ful-

ly-auto chemiluminescence immunoassay (CLIA) analyzer) were done in the macrocytic group. Serum Vitamin B12 values of less than 150 ng/L are a possible sign of a vitamin B12 deficiency.

Categorical variables are expressed as number of patients and percentage of patients and compared across the groups using two-tailed test of population proportion after deduction of z-score. Continuous variables are expressed as Mean  $\pm$  Standard Deviation and compared across the 2 groups using unpaired t test. The statistical software SPSS version 20 has been used for the analysis. An alpha level of 5% has been taken, i.e. if any p value is less than 0.05 it has been considered as significant.

### RESULTS

Among 100 participants, 47 were in the age group 18-50 year and 53 aged more than 50 year; 43 men and 57 women. 66 were having anaemia mostly normocytic normochromic (44) as shown in Diagram-1. Out of 19 cases of microcytic hypochromic anaemia 16 (84.2%) are found to be iron-deficient and out of 3 cases of macrocytic anaemia 2 (66.7%) are found to be having Vitamin B12 deficiency

Relation between prevalence of anaemia and different variables is shown in Table-1 and Table-2. Between 18 and 50 years of age prevalence of anaemia is higher among females (71.8%) than in males (53.3%) (p value=0.047). No such association was found among individuals  $>$  50years of age. Patients coming from rural (76.6%) backgrounds were found to have increased prevalence of anaemia than their urban (56.6%) counterparts (p value=0.003). Prevalence of anaemia increases progressively with increasing dosage of Metformin viz 500mg (Group A 50%), 1gm (Group B 58.53%) and 2gm/day (Group C 71.92%) (respective p values  $<$ 0.05).

**Table 1: Prevalence of anaemia with respect to age, sex and residence of subjects on Metformin for long duration**

Variables	Total	Anaemia (%)	P values
<b>Age</b>			
18-50y	47	31 (65.9)	0.5
$>$ 50y	53	35 (66)	
<b>Sex</b>			
Male	43	28 (65.1)	0.8
Female	57	38 (66.7)	
<b>Age with sex</b>			
Male 18-50y	15	8 (53.3)	0.04
Female 18-50y	32	23 (71.8)	
Male $>$ 50y	28	20 (83.3)	0.18
Female $>$ 50y	25	15 (60)	
<b>Residence</b>			
Rural	47	36 (76.6)	0.0003
Urban	53	30 (56.6)	

**Table 2:Prevalence of anaemia with respect to duration of drug use, daily dose and drug combinations used by subjects on Metformin for long duration**

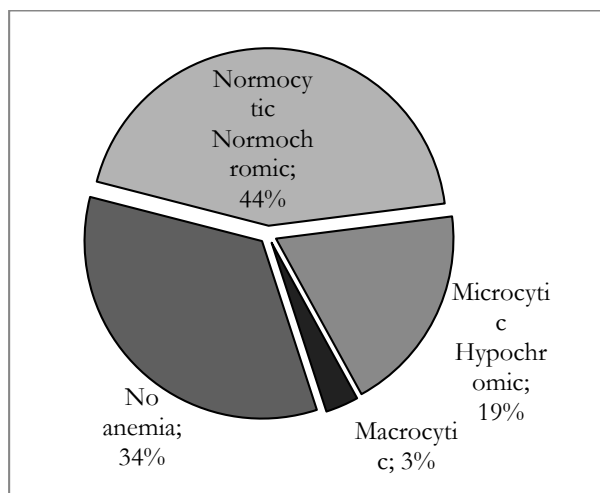
Variables	Total	Anaemia	P value
<b>Duration</b>			
≥5y and <10y	74	48 (64.9)	0.4
≥10y	26	18 (69.2)	
<b>Daily dose</b>			
500mg(A)	2	1 (50)	0.001(A & C)
>500mg & ≤1gm(B)	41	24 (58.5)	0.012(B & C)
>1gm & ≤2gm(C)	57	41 (71.9)	0.013(A & B)
<b>Drug combination</b>			
Metformin only(A1)	4	3 (75)	0.28(A1 & C1)
Metformin +Insulin(B1)	18	14 (77.8)	0.06(B1 & C1)
Metformin+OOHA (C1)	78	49 (62.8)	0.38(A1 & B1)

OOHA: Other Oral hypoglycaemic agents

**Table-3: Total leukocyte count(TLC) and platelet count among individuals with and without anaemia**

Variables	Anaemia(n=66) Mean ± SD	No anaemia(n=34) Mean ± SD
TLC(x10 <sup>9</sup> /μL)	9.68±3.21	9.32±3.29
Platelets(x10 <sup>5</sup> /μL)	2.51±0.99	2.38±1.02

P value for TLC 0.599; P value for platelet 0.540



**Figure 1: Pie diagram showing prevalence of anaemia and its types among study subjects**

Moreover there is negative correlation between Metformin dosage and haemoglobin levels ( $r \approx -1$ ) Nevertheless no such relation could be elicited between duration of Metformin use and anaemia i.e. between <10 years (64.9%) and ≥10 years (69.2%) (p value=0.4). Prevalence of anaemia was also more in individuals using insulin (77.8%) (B1) rather than other oral hypoglycaemic agents (62.82%) (C1) alongside Metformin (p value=0.04). No other statistically significant relation was found between Metformin use and other haematological parameters e.g. total leukocyte count (p value=0.59), platelet count (p value=0.54) etc. as evident from Table-3.

## DISCUSSION

Study by Cheekurthy A et al. has clearly demonstrated the rising trends of type 2 diabetes mellitus in Asian female population at present times due to reasons e.g. genetics, body fat distribution, sedentary lifestyle, gestational glucose intolerance etc.<sup>8</sup> In the present study also among 100 study subjects with type 2 Diabetes mellitus, females(57%) outnumber males(43%) by 14%.Prevalence of anaemia is more among females by a striking 18.5%(p value < 0.05) among the age group of 18-50 years but not so beyond 50 years. Women who are in their reproductive stage of life are vulnerable to several risk factors for anaemia e.g. frequent child birth, menstrual irregularities, malnutrition and infection as shown in study by Bentley M. E. et al.<sup>9</sup> An age cut-off of 50 year mostly marks the end of this stage. According to 2011 Indian Census data, rural and urban people account for 68.84% and 31.16% respectively of the total population i.e.121 crore. This massive rural population are deprived of access to health facilities, awareness, nutrition and social amenities. Therefore the present study shows rural individuals (76.6%) are found to be more anaemic than those having urban backgrounds (56.6%) notwithstanding the latter outnumbering their rural peers.

Our findings also conform to studies by D., Shailendra et al and Zalaket J et al showing rise in prevalence of anaemia with progressively increasing dosage of Metformin.<sup>10,11</sup> Study by Dujic T et al, 25% of patients using Metformin on long-term basis develop gastrointestinal adverse effects which compel at least 5% of them even to discontinue therapy .<sup>12</sup> The gastric intolerance and gastritis with increasing dosage might have caused malabsorption of micronutrients essential for hemopiesis. Contrary to eminent studies by Nervo M et al, our study does not show any significant relation between duration of Metformin therapy and prevalence of anaemia possibly due to long term diabetics had been excluded already for their end-organ micro and macrovascular complications.<sup>13</sup>However, our study shows the prevalence of anaemia to be more prevalent in patients using insulin (72.8%) compared to sulfonylurea(62.8%) along with Metforminby 10% (p value<0.05) which is contrary to popular study by Kang D et al.<sup>14</sup> Advanced disease process may itself result in anaemia and compel the individuals to use insulin instead of sulfonylurea at the same time. Many well acclaimed studies have shown Metformin use for long duration causes vitamin B12 deficiency but in this study macrocytic anaemia affects only 3% of the subjects and 66.7% of them shows decreased levels of vitamin B12.<sup>13,15,16,17</sup> This apparent paradox may be due to the dietary pattern in this region and use of traditional medicines containing vitamin supplements. The other types of anaemia seen are normocytic normochromic (44%) and microcytic hypochromic (19%)

with the latter mainly caused by iron deficiency (84.2%).

Detailed longitudinal follow-up, study of genetic, epigenetic factors and detailed introspect into aetiology of anaemia is needed to further validate these findings. As the baseline haematological status before starting Metformin is not available here, a longitudinal follow-up study is needed for further analysis.

## CONCLUSION

Physicians should be vigilant and advise regular screening for anaemia for patients with type 2 Diabetes Mellitus on long-term Metformin particularly young women below 50 years, people coming from rural backgrounds, individuals using increasing dosage of Metformin or insulin along with. Macrocytic anaemia, although less common than normocytic normochromic and microcytic hypochromic subtypes, is mainly caused by vitamin B12 deficiency which needs supplementation.

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