Original Article

COMPARATIVE STUDY OF VITAMIN B_{12} DEFICIENCY ASSOCIATED WITH METFORMIN AND METFORMIN IN COMBINATION WITH DPP-4 INHIBITORS IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Objective: To estimate the prevalence of vitamin B_{12} deficiency in type 2 diabetes mellitus patients receiving metformin and metformin in combination with DPP-4 inhibitors. To evaluate the vitamin B_{12} levels induced by long-term metformin usage.

Methods: It is a retrospective, comparative study of 6 mo, conducted at Shadan Institute of Medical Sciences, Hyderabad. 300 diabetic patients of age group between 30-85 y of either gender were divided into Group-A, receiving metformin and Group-B, receiving metformin+DPP-4 inhibitors. Ethics committee approval was o btained. The baseline and after 6 mo values of Vitamin B_{12} were noted and analyzed by using SPSS software.

Results: The majority of the patients were from the age group of 56-65 y (n=42, 28%) in Group-A and 46-55 y (n=61, 40.7%) in Group B. Male predominance was observed in both groups (n=81, 54% and n=76, 50.7%). Duration of Diabetes mellitus was ≤5 y in both groups (n=87, 58% and n=112, 74.7%). Vitamin B_{12} mean values for Group-A (Baseline-478.61, After 6 mo-195.94) and Group-B (Baseline-527.82, After 6 mo-299.05) were obtained. Mean reduction with a statistical significance in both study groups was observed (Group-A-282.66 and Group-B-228.77). Most of the patients showed numbness (14%) in Group-A and general weakness (7.3%) in Group B, respectively.

Conclusion: Type 2 diabetic patients who were on metformin therapy only have a prevalence of vitamin B_{12} deficiency compared to Metformin in combination with DPP-4 inhibitors receiving patients.

Keywords: Type 2 diabetes mellitus, Metformin, DPP-4 inhibitors, Vitamin B_{12}

INTRODUCTION

Type 2 diabetes mellitus has shown an estimated global impact of approximately 463 million in 2021 and is predictable to reach 700 million by 2045, according to the International Diabetic Federation (IDF) [1]. In a developing country like India, around 69.2 million people suffer from type 2 diabetes mellitus. Due to this, India has become the 2nd highest country next to China [2]. The prevalence of type 2 diabetes mellitus is expected to increase much further in developing as well as developed countries due to unhealthy lifestyles, which adds to the risk factors.

Type 2 diabetes mellitus comes with many risk factors, like obesity, general weakness, paresthesia, numbness, etc., in which vitamin B_{12} deficiency is also a major risk factor. In the general population of India, the reported vitamin B_{12} deficiency varies from approximately 12% to 67% [3, 4]. It can be said that a nutritionally unbalanced diet could be one of the major reasons for the increase in this major risk factor, vitamin B_{12} deficiency [5].

Vitamin B_{12} plays a chief role in the metabolism and is essential for the re-methylation of homocysteine to methionine. Its deficiency can lead to hyperhomocysteinemia, which has been related to macrovascular complications in type 2 diabetes mellitus patients [6]. Vitamin B_{12} deficiency in type 2 diabetes mellitus patients, if left untreated, can lead to hazardous consequences like neuropathy, anemia (megaloblastic), cognitive impairment, and memory weakening [7].

In type 2 diabetes mellitus patients who are on Metformin, Metformin-induced vitamin B_{12} deficiency has been reported in the past by the Indian population [8]. In the literature, there are fewer studies regarding an association between Metformin treatments singly as well as in combination with DPP-4 inhibitors in T2DM and low serum vitamin B_{12} levels in the Indian population.

The present study has been taken up with the intent to evaluate the relationship between Vitamin B_{12} levels in type 2 diabetic patients who are consuming oral doses of Metformin singly as a treatment strategy as well as in combination with DPP-4 inhibitors.

MATERIALS AND METHODS

Study design

It is a retrospective, comparative study of six months conducted at the Shadan Institute of Medical Sciences, Hyderabad. A total of 300 participants were enrolled in the study suffering from type 2 diabetes mellitus disorder. The study comprised patients of either gender, aged between 30 to 85 y. The approval to conduct this study was obtained from the Institutional Ethics Committee (IEC) of the Shadan Institute of Medical Sciences and the teaching hospital with the reference number. [Ref. No. 012/SIMS/Research/2022 and written informed consent were obtained from all the participants before the initiation of the study.

The study groups were divided into Group A, which received an oral dose of Metformin (n=150), and Group B, which received an oral dose of Metformin in combination with DPP-4 inhibitors (n=150).

Patients with type 2 diabetes mellitus of duration ≥ 5 y and ≥1 0 y aged between 30 to 85 y, of either gender, who were on Metformin based oral hypoglycemic agents' therapy were included in the study. Non-diabetic patients with an age>85 y and with comorbidities like hypothyroidism, cancer, etc. and those who were on insulin therapy were excluded from the study.

Study procedure

A retrospective chart review was done for patients seen by a provider. Using search queries of each medical record, type 2

diabetic patients between 30-85 y of age with an active prescription for oral hypoglycemics with Metformin were recorded. It was decided to look back at, the last five-year records in this study.

Patient demographic information (age, gender, and duration of diabetes mellitus), as well as laboratory data, were gathered (Vitamin B_{12} levels). Low and inadequate vitamin B_{12} readings made up a major fraction of the sample.

Statistical analysis

The demographic data obtained were subjected to descriptive statistical analysis, and by using SPSS software, the data is stated as mean±SD, frequencies (n), and percentages (%) in tabulated and graphs form. Student t-test was performed to test the significance of the difference between the means of the study groups. In all the cases, p value ≤0.05* is considered statistically significant.

RESULTS

A total of 300 type 2 diabetic patients were enrolled in the present study. Out of 300 patients, 150 were on oral therapy Metformin (Group-A) and the remaining 150 were on Metformin in combination with DPP-4 inhibitors (Group-B).

Most of the patients were aged between 56-65 y (n=42, 28%) in group-a and in group-b, the majority (n=61, 40.7%) were from the age group 46-55 years. A mean age of 58 y in Group-A and 50 y in Group B was noted (table 1 and fig. 1).

Male predominance was noticed in both Group-A (n=81, 54%) and Group B (n=76, 50.7%), respectively. (Mean values, Group-A-1.46 and Group-B-1.49) (Table 1 and fig. 2).

When the duration of Diabetes mellitus was determined, Group-A (n=87, 58%) and Group-B (n=112, 74.7%), both showed a majority in ≤ 5 years of diabetic history, respectively.

A mean duration of 8.57 y in Group-A and 6.71 y in Group B was observed (table 1 and fig. 3).

Vitamin B_{12} levels were recorded at baseline and after 6 mo in each study group. In Group-A (Metformin), baseline levels were in a high percentage (n=112, 74.7%) at the normal range (>350pg/ml), and after 6 mo of study, the Vitamin B_{12} levels in the Metformin study group were at low range (<211 pg/ml) in the majority (n=49, 66%) (table 2 and fig. 4).

In Group-B (Metformin+DPP-4 inhibitors), baseline levels were in a high percentage (n=115, 76.7%) at the normal range (>350pg/ml), and after 6 mo of study, the Vitamin B_{12} levels in Metformin+DPP-4 inhibitors study group were at Borderline range (211-350 pg/ml) in the majority (n=58, 38.7%) (table 2 and fig. 4).

A mean reduction in study groups was noted as well. A mean reduction with statistical significance in both study groups was observed. In Group-A, the mean value of Vitamin B_{12} at baseline was 478.61, which after 6 mo was 195.94. A mean reduction of 282.66 was noted. In Group B, the mean value of Vitamin B_{12} at baseline was 527.82, which after 6 mo was 299.05. A mean reduction of 228.77 was noted (table 3 and fig. 5).

Symptoms of Vitamin B_{12} deficiency were also recorded in both study groups. Group-A records showed numbness (14%) in the majority of the patients, and in Group B most of the patients had general weakness (7.3%) (fig. 6).
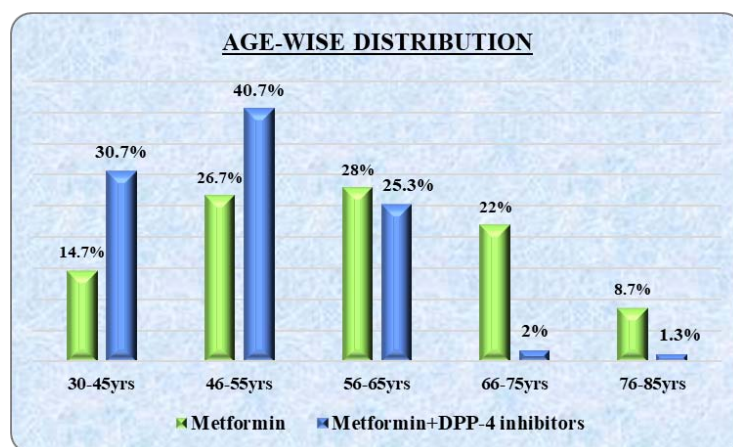
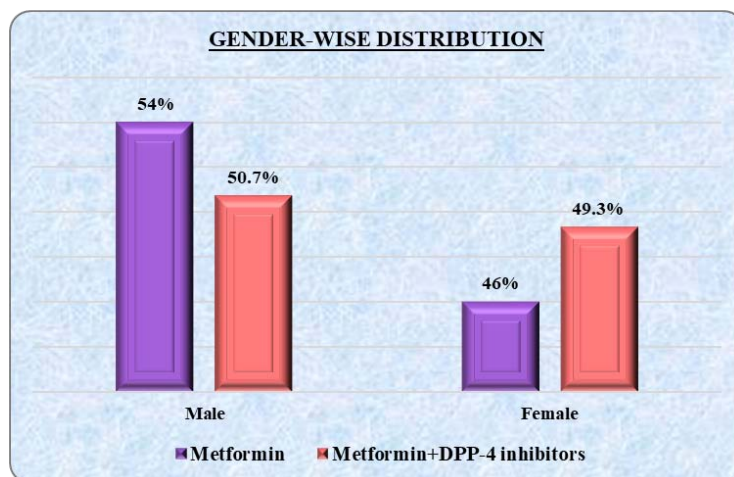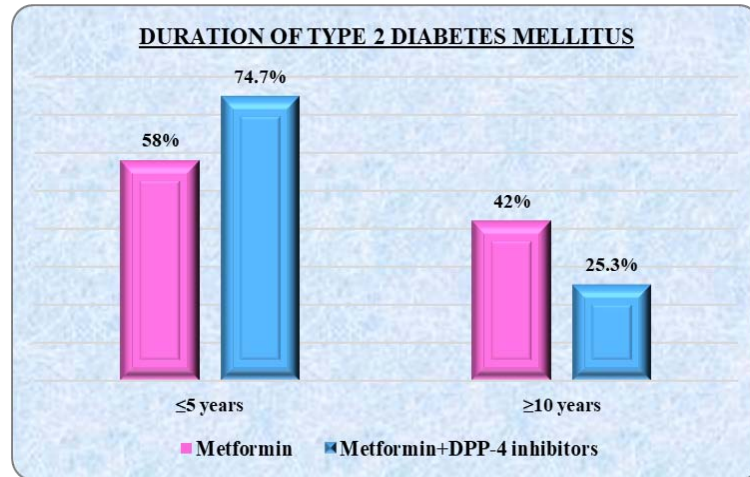

Fig. 1: Age-wise distribution

Fig. 2: Gender-wise distribution

Table 1: Demographic data

Study variables	Group A (Metformin)	Group B (Metformin+DPP-4 inhibitors)
	Mean±SD	Mean±SD
Age (30-85 y)	58.98±12.2	50.63±9.10
Gender (M/F)	1.46±0.50	1.49±0.50
Duration of type 2 diabetes mellitus (≥5 y/≥10 y)	8.57±6.70	6.71±4.77

Fig. 3: Duration of type 2 diabetes mellitus

Table 2: Vitamin B$_{12}$ values in study groups

Vitamin B$_{12}$	Metformin		Metformin+DPP4	
	Frequency (n=150)	Percentage (%)	Frequency (n=150)	Percentage (%)
Baseline				
Normal (>350pg/ml)	112	74.7%	115	76.7%
Borderline(211-350 pg/ml)	35	23.3%	34	22.7%
Low(<211 pg/ml)	03	2%	01	0.7%
After 6 mo				
Normal (>350pg/ml)	10	6.7%	51	34%
Borderline(211-350 pg/ml)	41	27.3%	58	38.7%
Low(<211 pg/ml)	49	66%	41	27.3%
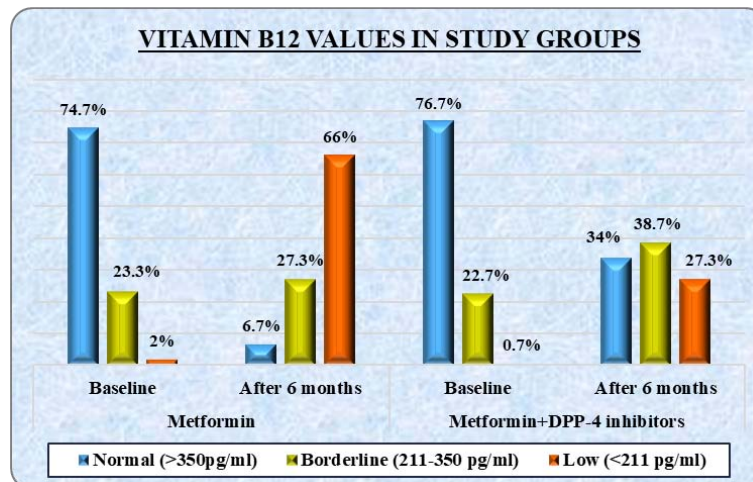
Fig. 4: Vitamin B$_{12}$ values in study groups

Table 3: Mean values of vitamin B$_{12}$ in study groups

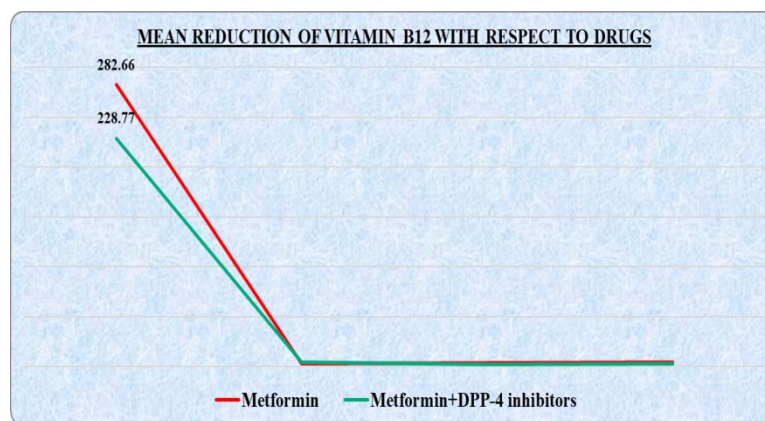| Study drugs	Vitamin B$_{12}$ (Baseline)	Vitamin B$_{12}$ (After 6 mo)	Mean reduction	p value
Metformin	478.61±195.02	195.94±149.10	282.66±163.26	0.001*
Metformin+DPP4	527.82±191.55	299.05±125.17	228.77±220.28	0.001*

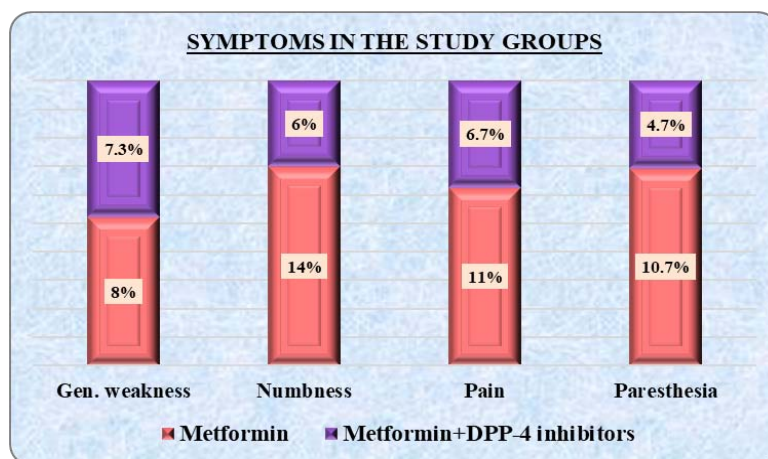**Fig. 5: Mean reduction of vitamin b12 concerning drugs**

Fig. 6: Symptoms in the study groups

DISCUSSION

In the present study, in Group-A, most of the patients were aged between 56 to 65 y (n=42, 28%), and in Group B, the majority (n=61, 40.7%) were from the age group 46-55 y. A mean age of 58 y in Group-A and 50 y in Group B was noted.

It is as per a study done by Yashaswini L. S. in 2019, where the majority of the type 2 diabetes patients were in the 50 to 55 y of age group [9]. It is also similar to a study done on T2DM patients by Raizada N *et al.*, in 2017, where the predominant age group was between 51 to 8 y [10].

Male predominance was noticed in both Group-A (n=81, 54%) and Group B (n=76, 50.7%), respectively. (Mean values, Group-A-1.46 and Group-B-1.49).

In the studies done by Elsaier A *et al.*, in 2017, and by Lhadd T *et al.*, in 2018, male type 2 diabetic patients were in the majority compared to females with vitamin B_{12} deficiency [11, 12].

When the duration of diabetes mellitus was determined, Group-A (n=87, 58%) and Group-B (n=112, 74.7%), both showed a majority in ≤ 5 years of diabetic history, respectively.

A mean duration of 8.57 y in Group-A and 6.71 y in Group B was observed.

It is similar to a study done by Agarwal P *et al.*, in 2016, where the mean duration of T2DM was around 6.15 y [13].

Vitamin B12 levels were recorded at baseline and after 6 mo in each study group. In Group-A (Metformin), baseline levels were in a high percentage (n=112, 74.7%) at the normal range (>350pg/ml), and after 6 mo of study, the Vitamin B12 levels in the Metformin study group were at low range (<211 pg/ml) in the majority (n=49, 66%).

In Group-B (Metformin+DPP-4 inhibitors), baseline levels were in a high percentage (n=115, 76.7%) at the normal range (>350pg/ml), and after 6 mo of study, the Vitamin B12 levels in Metformin+DPP-4 inhibitors study group were at the borderline range (211-350 pg/ml) in the majority (n=58, 38.7%).

The above results are similar to a study done by Khan F *et al.*, in 2021, where their study showed a high prevalence of vitamin B_{12} deficiency in type 2 diabetic patients consuming Metformin and an association between the Metformin dose (oral) and Vitamin B_{12} levels [14].

A mean reduction in study groups was noted as well. A mean reduction with statistical significance in both study groups was observed. In Group-A, the mean value of Vitamin B12 at baseline was 478.61, which after 6 mo was 195.94. A mean reduction of 282.66 was noted. In Group B, the mean value of Vitamin B12 at baseline was 527.82, which after 6 mo was 299.05. A mean reduction of 228.77 was noted.

These results coincide with a study done in 2016 by Damiao CP *et al.*, where T2DM patients on single Metformin therapy have more chances of developing Vitamin B_{12} deficiency [15].

Symptoms of Vitamin B12 deficiency were also recorded in both study groups. Group-A records showed numbness (14%) in the majority of the patients and in Group B most of the patients had general weakness (7.3%).

Similar results were noticed in a study done by Nagaraj Kotli *et al.*, in 2019, where general weakness was observed in T2DM patients with Vitamin B12 deficiency on Metformin treatment [16].

LIMITATIONS OF THE STUDY

The small sample size and short duration of study time were the major limitations of our study. The study could have been extended

with a large sample size to confirm our findings, which again emerged as a limitation of our study.

CONCLUSION

In our study, it can be concluded that type 2 diabetic patients who were on only Metformin therapy (monotherapy) have a greater prevalence of vitamin B_{12} deficiency compared to the type 2 diabetic patients who were on Metformin in combination with DPP-4 inhibitors. Hence, Metformin in combination with DPP-4 inhibitors is a better choice of drug combination for type 2 diabetic patients concerning vitamin B_{12} deficiency.

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AUTHORS CONTRIBUTIONS

Significant contributions have been made by all authors in data collection, statistical analysis, writing, editing, reviewing, and submitting the manuscript for publication. Nabiha Subhani Misbah suggested the topic, monitored the study, and helped in manuscript writing, and Syeda Ayesha Siddiqua helped with statistical analysis. Juwaria Masood helped in data collection and compilation. Naser Ashraf Tadvi helped with manuscript writing, editing, and reviewing. Arooba Fatima helped in data collection and manuscript review.

CONFLICTS OF INTERESTS

Declared none

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