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Original Research Article

Effect of metformin on serum vitamin B12 and red blood cell indices in North Indian subjects with diabetes mellitus

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ABSTRACT

Background: Diabetes mellitus (DM) affecting almost half a billion people worldwide and India is amongst the top ten countries of adults with diabetes. Metformin, the first-line therapy for diabetes, is associated with vitamin B12 malabsorption and subsequently, the development of vitamin B12 deficiency/insufficiency could manifest severe complications like neuropathy or anemia in the future. This study evaluated the effect of metformin on vitamin B12 and RBC indices in the North Indian population.
Methods: This study was executed at a tertiary care hospital. 35 T2DM (type 2 DM) participants with ongoing metformin therapy were compared with 27 T2DM participants without metformin therapy. Participants were recruited from outpatient after diagnosis as per American diabetes association (ADA) criteria.
Results: Metformin-treated participants had significantly low hemoglobin (t=2.096, df=60, 0.0403) compared to untreated participants. Similarly, MCHC was significantly lower in the metformin group (mean=33.28 gm/dl) compared to non-metformin group (mean=34.53 gm/dl) (t=2.745, df=60, p=0.0080). Moreover, there was a strong negative correlation (r=-0.4613, p=0.0053) among vitamin B12 and MCV in metformin group. There was no statistically significant correlation between vitamin B12 and RBC indices (MCV, MCH, MCHC) in the non-metformin group. Analyzing contingency table (Fisher's exact test), we found no major difference (p=0.2002) between two groups of vitamin B12 with an odds ratio of 2.026 (95% CI=0.7366 to 5.633). Unpaired t test also confirmed insignificancy (t=0.04077, df=60, p=0.9676).
Conclusions: Strong negative correlation was observed between vitamin B12 and MCV. Despite the insignificant difference of vitamin B12 between metformin and non-metformin groups, significantly low MCHC was found in metformin-treated participants.

Keywords: Metformin, RBC indices, Type 2 diabetes mellitus, Vitamin B12 deficiency

INTRODUCTION

Diabetes mellitus (DM) is one of the fastest-growing health emergencies in the 21st century and so far, approximately half a billion people are living with diabetes across the globe. According to the International diabetes federation (IDF), recently India ranked 2nd amongst the top ten countries/territories for the number of adults (20-79 years) with diabetes in 2019.[1] DM is an endocrinopathy characterized by insufficient or absolute lack of insulin secretion, insulin resistance and consequently progression of microvascular and macrovascular complications along with altered fuel metabolism, which ultimately reduces overall life expectancy and quality. Prevention and management of diabetes become a significant health challenge.

Metformin is an oral antihyperglycemic agent and is the first-line pharmacologic treatment for diabetes worldwide according to recent guidelines of ADA. Metformin is the first choice because it reduces the chance of overt hypoglycemia with minimal adverse effects, mainly gastrointestinal. Metformin (1,1-dimethyl biguanide) is the only biguanide widely used to treat T2DM worldwide.[2] Biguanides are derivate that has the blood glucose-lowering property in T2DM patients. As biguanides do not cause overt hypoglycemia by increasing plasma insulin levels, these are generally termed antihyperglycemic rather than hypoglycemic agents.[3] Metformin lowers blood glucose levels and enhances insulin sensitivity.[4] Metformin has gained popularity for pleiotropic effects. Studies showed decreased food intake and reduced body weight are associated with metformin therapy.[4,5] Research also indicated its action beyond T2DM to gestational DM, polycystic ovarian syndrome, metabolic syndrome and diabetes prophylaxis.[6] This drug reduces cardiovascular and possibly cancer risk.[7]

Vitamin B12 malabsorption associated with short-term metformin therapy in patients with DM was established for the first time by Berchtold et al in 1969 and later confirmed by Tomkin et al in 1971 with large sample size and long-term metformin therapy.[8,9] Several studies have been conducted afterward to support the association between vitamin B12 deficiency and metformin. Although the manifestation of vitamin B12 deficiency associated with metformin therapy takes a longer time, it could develop severe complications like neuropathy, megaloblastic anemia if left untreated.[10] Due to the fewer data on metformin-induced vitamin B12 deficiency or insufficiency from Northern India, we conducted this study to evaluate the vitamin B12 status in T2DM subjects and the risk factors associated with it.

METHODS

This cross-sectional pilot study was performed in a tertiary care hospital between January 2018 and November 2019. The study was carried out in the department of medicine collaboration with the Rajiv Gandhi centre for diabetes and endocrinology and the department of pathology, J N medical college, AMU, Aligarh, India. Necessary clearance was obtained from institutional ethical committee.

Study subjects

A total of 62 T2DM patients of both sexes regularly visited the hospital constituted the sample size for the study. Data were collected using questionnaires and bio-information conducted in the outpatient department of Rajiv Gandhi centre for diabetes and endocrinology and the department of medicine. Informed consent was obtained from each subject before the initiation of the study. The patients were divided into two groups (metformin treated=35 and untreated=27) depending on the drug administration. The diagnosis of diabetes was based on the current updated criteria of the ADA. Metformin group comprised of T2DM patients of ongoing metformin treatment with a duration of metformin use ≥3 months while non-metformin group consisted of T2DM patients who had never received metformin or other oral antidiabetic drugs and on dietary modification. History of diabetes onset, dose and duration of metformin usage was documented for each patient.

Inclusion criteria

Participants were defined as type 2 diabetic according to the ADA criteria with age above 30 years at the time of diagnosis. Patients with fasting blood glucose ≥126 mg/dl on two different occasions, postprandial ≥200 mg/dl or HbA1c ≥6.5% considered for the diagnosis of diabetes. Type 2 diabetic subjects who intake metformin ≥3 months/other oral antidiabetic drugs/dietary modifications were included in the study. Informed consent from each participant was obtained after explaining the study purpose and the procedures in their local languages. Participants were assured of keeping their information confidential and advised to join voluntarily.

Exclusion criteria

Type 1 and type 2 DM on insulin therapy, strictly on the vegetarian diet, multiple complications of T2DM and overt diabetic, kidney liver and thyroid diseases, alcohol abuse and family history of peripheral neuropathy, malabsorption syndrome, partial/total gastrectomy, pernicious anemia, hematological diseases, patients on oral/intravenous (IV) vitamin B12 supplements or multivitamins, over the counter medications, calcium supplements, histamine-2 blocker, proton pump inhibitor and in case of non-consent were excluded.

Sample collection

Blood samples were collected following aseptic precautions by venipuncture. Each blood sample was divided into two parts. The first one was collected in EDTA containing vial to perform complete blood count (CBC), another part in the plain vial for HbA1c estimation and vitamin B12 assay. Serum samples (centrifuged at 1000 g for 15 min) stored at minus 80 °C for a maximum of 7 days till assayed for vitamin B12. Although the stability of the serum vitamin B12 lasts for longer than one week while refrigerated, we performed the vitamin B12 assay within seven days after sample collection.[11]

Biochemical parameters

The serum vitamin B12 level was estimated by the chemiluminescence immunoassay method following standard protocol using Beckman Coulter Access2 (USA) analyzer. WHO defined serum vitamin B12 level of <150 pg/ml was considered deficient. For this study, 200-300 pg/ml as borderline and above 300 pg/ml as normal (up to

900 pg/ml). Glycated haemoglobin (Hb) was assayed by high-performance liquid chromatography (HPLC) method using Premier Hb9210 (Trinity Biotech). The reference interval of HbA1c (%) was considered per ADA criteria for the diagnosis of diabetes.

Pathological parameters

Hb, Hematocrit (HCT), Red blood cell (RBC) count and RBC indices like Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC) were determined using MICROS ABX automated analyzer according to the manufacturer's protocol.

Statistical analysis

Data entered on Microsoft excel spreadsheets and analyzed by GraphPad Prism software.[8] Differences between means compared by unpaired t test and descriptive statistics of each variable were calculated. Pearson's correlation was performed to determine the strength of correlation between vitamin B12 and different erythrocyte indices. Fisher's exact test was performed for contingency table analysis. Results expressed as mean with 95% confidence interval (CI). For all analyses, $p<0.05$ was considered statistically significant.

RESULTS

Mean age was higher in the metformin group (59.40±7.655 SD) compared to the non-metformin group (52.89±8.382 SD) with a p value of 0.0023 and 95% CI of -10.60 to -2.423. Duration of diabetes predominance was observed in the metformin group ($p<0.0001$). Hb concentration was significantly lowered in the metformin group and the mean difference was statistically significant ($t=2.096$, $df=60$, $p=0.0403$) when compared by unpaired t test with a 95% CI of 0.03779 to 1.609. Table 1 shows the basic characteristics of the two studied groups.

Analyzing the data by contingency table (Fisher's exact test), we found no major difference ($p=0.2002$) between two variables of vitamin B12 with an odds ratio of 2.026 (95% CI 0.7366 to 5.633). Unpaired t test also confirmed about non-significance ($t=0.04077$, $df=60$, $p=0.9676$) at 95% CI (-85.12 to 88.66). In addition, mean MCHC was low in both the cases of metformin (33.28 gm/dl) and non-metformin (34.53 gm/dl) group and significantly different ($t=2.745$, $df=60$, $p=0.0080$). MCV and MCH between both the groups are compared and found no significant difference (Figure 1).

Table 1: Baseline characteristics of metformin and non-metformin group.

Characteristics	Metformin (n=35)	Non-metformin (n=27)	P value (two tailed)	95% CI
Age (years)	59.40 (±7.655)	52.89 (±8.382)	0.0023	-10.60 to -2.423
Sex				
Male N (%)	16 (45.7 %)	19 (70.4 %)		
Female N (%)	19 (54.3 %)	8 (29.6 %)		
Duration of diabetes	8.686 (±2.272)	3.963 (±1.454)	<0.0001	-5.727 to -3.719
HbA1c (%)	8.597 (±1.483)	7.315 (±1.084)	0.0004	-1.961 to -0.6037
Hb (gm/dl)	10.72 (±1.426)	11.54 (±1.664)	0.0403	0.03779 to 1.609
HCT (%)	32.21 (±4.244)	33.49 (±4.956)	0.2770	-1.056 to 3.623
RBC count (million/mm^3)	3.575 (±0.5295)	3.774 (±0.5388)	0.1507	-0.0744 to 0.472

HbA1c: glycated haemoglobin; Hb: hemoglobin; HCT: hematocrit, RBC: red blood cell.

Table 2: Pearson's correlation and linear regression of vitamin B12 with red cell indices (MCV, MCH, MCHC) in metformin and non-metformin groups.

Groups	Pearson's correlation				Best-fit values (linear regression)		
	r	R squared	95% CI	P value	Slope	Y-intercept	X-intercept
Metformin (n=35)							
MCV	-0.4613	0.2128	-0.6887 to -0.1513	0.0053	-0.02263	99.17	4383
MCH	-0.3019	0.09115	-0.5771 to 0.03484	0.0780	-0.005714	32.41	5672
MCHC	0.06167	0.003804	-0.2773 to 0.3870	0.7249	0.0006861	33.02	-48128
Non-metformin (n=27)							
MCV	-0.2143	0.04594	-0.5496 to 0.1804	0.2830	-0.009214	93.74	10174
MCH	-0.1325	0.01757	-0.4880 to 0.2606	0.5099	-0.001452	31.26	21530
MCHC	0.2571	0.06609	-0.1362 to 0.5804	0.1955	0.002514	33.57	-13352

MCV=mean corpuscular volume, MCH=mean corpuscular haemoglobin, MCHC=mean corpuscular haemoglobin concentration, r=correlation coefficient.

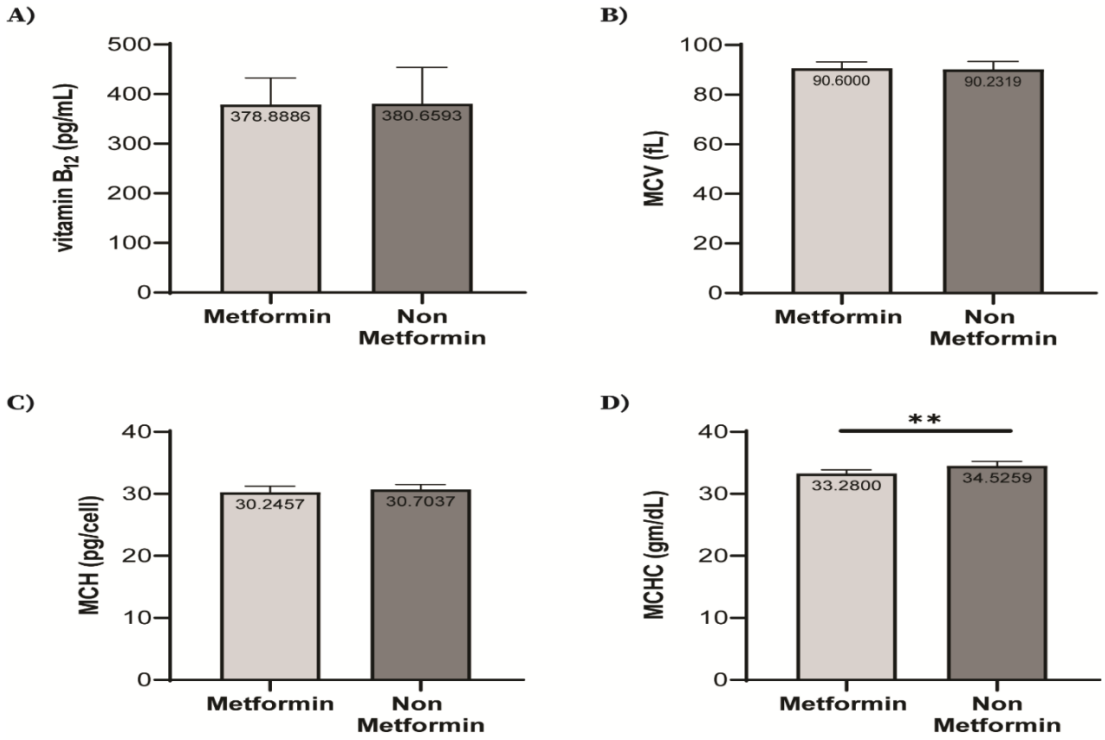

Figure 1: Means of (A) vitamin B12, (B) MCV, (C) MCH and (D) MCHC between metformin and non-metformin groups are compared using an unpaired t test; means of MCHC are significantly different from each other.

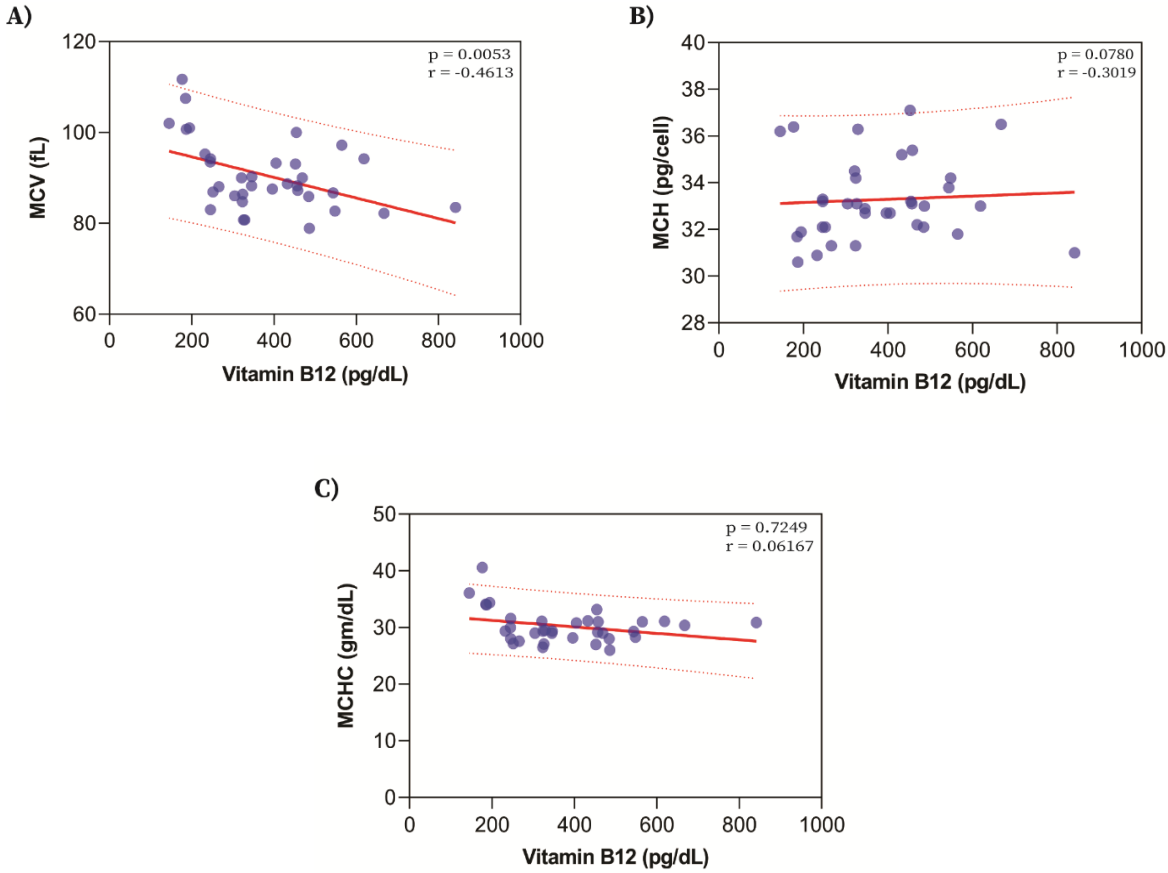

Figure 2: Correlations of vitamin B12 with (A) MCV, (B) MCH and (C) MCHC among metformin treated group.

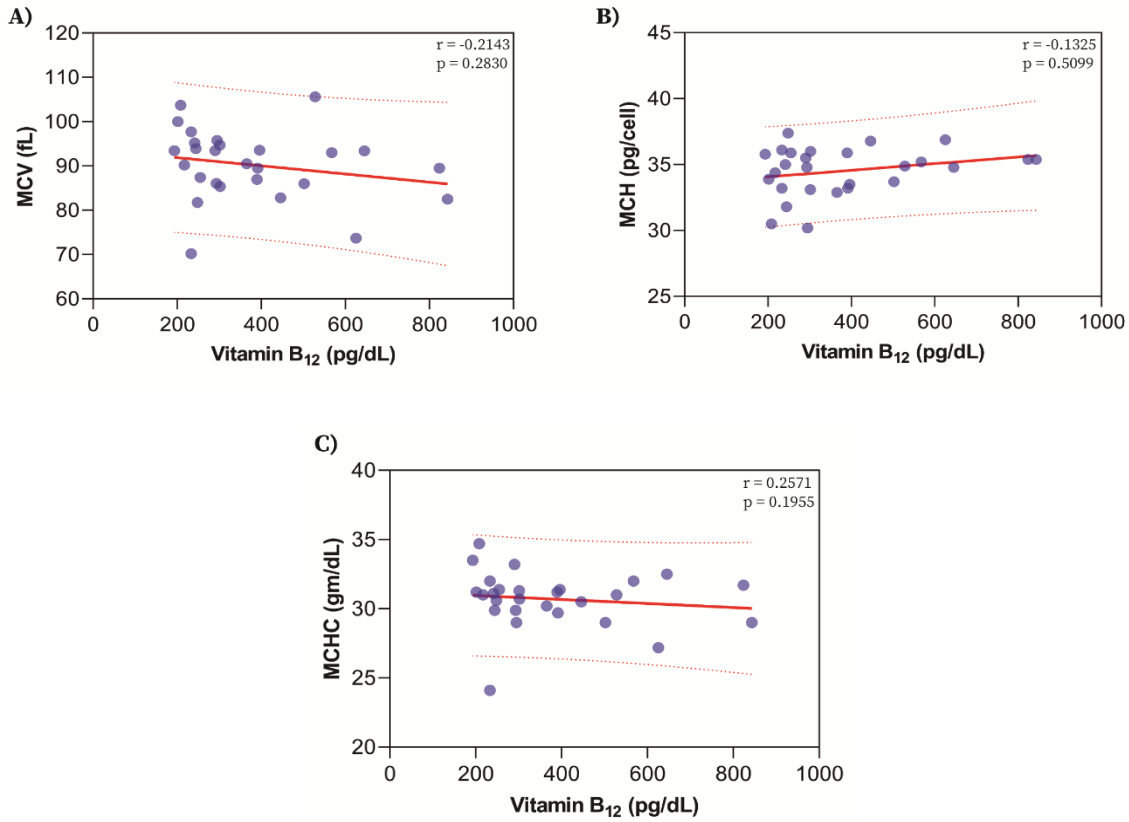

Figure 3: Correlations of vitamin B12 with (A) MCV, (B) MCH and (C) MCHC among non-metformin group.

Moreover, there was a strong negative correlation (r=-0.4613, p=0.0053) among vitamin B12 and MCV in metformin group (Figure 2).

There was no statistically significant correlation between vitamin B12 and RBC indices (MCV, MCH, MCHC) in the non-metformin group (Figure 3).

Table 2 shows Pearson's correlation and linear regression of vitamin B12 with RBC indices (MCV, MCH, MCHC) in the metformin and non-metformin groups.

DISCUSSION

According to ADA, metformin should be the initial pharmacologic agent in combination with lifestyle modifications when T2DM was diagnosed.[12] Metformin primarily acts on complex I of the Electron transport chain (ETC) within mitochondria and the resulting fall of energy charge leads to the activation of the 5' AMP-activated protein kinase (AMPK) and inhibition of glucagon induced cAMP pathway.[13-15] Both the pathways effectively reduced glucose in different tissues. Metformin hinders the calcium-dependent absorption of vitamin B12-intrinsic factor complex at the terminal ileum.[16] The probable cause of this inhibition might be due to the repelling of other cations (Ca^{2+}) by the highly accumulated positively charged metformin (at physiological pH) around the plasma membrane. Elevated blood homocysteine increases the susceptibility to cardiovascular disease (CVD). Folate and vitamin B12 were among the crucial nutrients involved in homocysteine metabolism and maintain blood homocysteine concentration efficaciously.

Metformin-induced vitamin B12 deficiency or insufficiency causes hyperhomocysteinemia, which is independently related to CVD and retinopathy.[17,18] Some studies have found unchanged blood homocysteine even with decreased vitamin B12 in individuals on metformin therapy.[18-20] On the contrary, many studies demonstrated homocysteine rise despite the metformin-induced vitamin B12 diminution.[16,21] The discrepancy among these studies indicates that additional factors like age, nutritional status, food habits, metformin dosage are accountable for metformin-induced vitamin B12 reduction.

Metformin dosage might be one of them. In Japanese individuals, a metformin dosage of >1.2 g could cause vitamin B12 deficiency/insufficiency.[18] We also observed the same in the Northern Indian population where the majority of the patients with serum vitamin B12 deficiency had a metformin dose of higher than >1 g per day, whereas most of the patients displayed normal vitamin B12 when the metformin dose was <1 g. Age is another important factor for this discrepancy since older people are more prone to develop vitamin B12 deficiency or insufficiency.[22] There is no strong correlation has been found between vitamin B12 and the duration of diabetes in our study. A study was also conducted on the northern

Indian T2DM population, but no strong correlation had been found between vitamin B12 level and duration of metformin use.[23] In this present study, low MCHC and increased MCV in patients on metformin therapy indicate the development of macrocytic anemia in later stages.

The limitation of this study was that we were unable to measure other metabolites related to vitamin B12 such as homocysteine, methylmalonic acid. Additionally, small sample size was considered for this study.

CONCLUSION

Despite the nonsignificant difference in serum vitamin B12 levels between metformin-treated and non-treated T2DM patients, metformin therapy causes asymptomatic vitamin B12 deficiency/insufficiency in these patients, which is clinically significant. Moreover, the noteworthy difference of MCHC between the two groups and the strong negative correlation of vitamin B12 with MCV in metformin-treated patients implies that metformin therapy might interfere with MCV and MCHC if subjected to further studies. Hence regular screening of serum vitamin B12 along with erythrocyte indices is suggestive of managing T2DM in a better way who are on metformin therapy. Research is required to evaluate the long-term effect of high-dose metformin administration in the Indian population. Due to different ethnic backgrounds, epigenetic status can also be explored to understand the root cause of metformin-induced vitamin B12 deficiency. Link can also be established between gene polymorphism and metformin-induced vitamin B12 deficiency if explored.

The implementation of routine universal screening would be expensive for the public healthcare system. Therefore, identifying all these probable risk factors associated with developing vitamin B12 deficiency or insufficiency would be a better way to empower the screening of susceptible patients on metformin therapy in the future.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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