The impact of long term use of hypoglycemic drugs and its association in the risk of osteoporosis, cardiovascular and mineral abnormalities in type 2 diabetes mellitus.

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ABSTRACT:
Introduction: Diabetes mellitus is a group of metabolic disorders that results in hyperglycemia due to insulin deficiency, impaired insulin action or both. This study was intended to determine the status of parameters related to blood glucose, homocysteine, serum magnesium, serum zinc and vitamin D in type 2 diabetes mellitus patients and compare with that of control. **Methods:** The study was conducted on 40 type 2 diabetes mellitus patients of 60-70 age and 40 matched healthy controls. Fasting sample was taken from each patient and control and analyzed for glucose, homocysteine, Zinc, magnesium and vitamin D. **Results and conclusion:** The observation of the present study reveals that there was a relation between the plasma glucose, Serum homocysteine, Serum Zinc, Serum magnesium and Serum vitamin D and the hypoglycemic drugs (mainly Metformin) in the long term treatment of type 2 diabetes mellitus. The clinical findings of the present study show that there was an increase in homocysteine level and decrease in the level of vitamin D, serum magnesium and serum zinc in patients taking hypoglycemic drugs for long-term. Therefore, it is clear that clinical and experimental evidence proves that hypoglycemic drug plays a major role in changing many physiological conditions. The wide spread application of hypoglycemic drugs has increased the risk of osteoporosis, cardiovascular changes and mineral abnormalities.
Keywords: Diabetes, homocysteine, Zinc, magnesium and vitamin D

INTRODUCTION: Diabetes mellitus is a metabolic disorder due to insulin deficiency and inefficiency resulting hyperglycemia. In 2019, about 9.3% of global population suffers from this disease and it is in steep rise [1, 2]. Diabetes causes serious health complications including renal failure, heart disease, stroke, and diabetic neuropathy etc. [3]. Vitamin D, Zinc and Magnesium plays a crucial role in mineral homeostasis. Vitamin D mainly responsible for skeletal health, its deficiency classically leads to rickets in children and osteomalacia in adults. The hypovitaminosis D associated with diabetes and vascular complications as it may influence insulin secretion and sensitivity [4]. The vitamin D deficiency also leads to elevation of homocysteine that causes osteporosis and vascular complications [5]. Increased homocysteine levels involved in epithelial damage and this is more strongly associated with type 2 diabtes [6].

However some other studies reporting that the homocysteine levels are normal or both lower and higher in diabetes [7-9].

The trace elements like zinc and magnesium function as cofactors for majority of the metabolic activities. Magnesium may contribute to the pathogenesis of diabetes associated complications due to losses of magnesium from gastrointestinal (GI) tract or kidneys. This can be seen in long term usage of hypoglycemic drug administration [10]. Zinc plays an important role in the maintenance of several tissue functions and has antioxidant property. Zinc is involved in the production, retention, and release of insulin. Zinc enhances the effectiveness of insulin in vitro and it has been postulated that zinc deficiency may aggravate the insulin resistance in non-insulin dependent diabetes [11, 12]. Hyperglycemia from type 2 diabetes mellitus causes physiologically significant losses of zinc from the body. Lower serum zinc levels were found to be responsible for the development of macrovascular complications in type-2 diabetics [12]. However, studies on zinc and magnesium supplementation with regard to glycemic control in diabetes mellitus had given beneficial results [14, 15].

Biguanides (such as metformin) are the most widely prescribed first-line treatment for type 2 diabetes. These medications reduce glucose production from the liver (most likely by inhibiting gluconeogenesis). The biguanides are derivatives of the chemical biguanide (guanylguanidine) that reduce blood glucose levels in people with type 2 diabetes. Biguanides reduce hepatic glucose synthesis, enhance peripheral glucose absorption, and moderately lower LDL and triglyceride levels. Hypoglycemic agents, such as biguanides, are responsible for clinical complications in type 2 diabetes, including cardiovascular disease [11,12] .

The current study is intended to provide the necessary analytical information regarding the correlation of fasting blood sugar (FBS), Vitamin D, Homocysteine, serum magnesium and serum zinc in patients with type 2 diabetes mellitus and to establish the relationship between long term use of hypoglycemic drugs and its associated risk factors.

MATERIAL AND METHODS: This case-control study was conducted at Madhubani Medical College (MMC), Madhubani, Bihar, with the agreement of the Institutional Ethics Committee. (Letter No. IEC/MMC/3155/04/22, dated 30/05/2022). Informed consent was obtained from all age and gender-matched individuals in the appropriate groups. The participants were separated into two groups: Control (n=40) and Cases who is on hypoglycemic drug treatment (n=40). The study focused on people aged 40 to 70.

Study population: A sample of 80 participants from the outpatient (OPD) and inpatient (IPD) departments were obtained from Madhubani Medical College, Madhubani.

Inclusion criteria: The individuals with established instances of type 2 diabetes mellitus taking hypoglycemic drugs.

Exclusion criteria: individuals with renal failure, malignancies, pregnant or lactating women, and participants who were unwilling to take part in the study.

Sample collection and estimation of biochemical parameters : All the participants underwent a thorough clinical examination, with age, sex, family history, and clinical history all being documented. Following an 8-hour overnight fast, aseptic precautions were taken to draw 5ml of venous blood from the ante-cubital vein of all the subjects. 5 ml of venous blood will be divided into 2ml in a fluoride oxalate vial for fasting plasma glucose estimation via Glucose Oxidase-Peroxidase (GOD-POD) method and 2ml in an acidic citrate vial for total plasma homocysteine estimation via stable isotope dilution and liquid chromatography electrospray tandem mass spectrometry (LC-MS/MS) method. Then, 1 ml in a plain vial will be for serum Vitamin D

estimation via chemiluminescence immunoassays (CLIA), serum Magnesium estimation via Calmagite method and serum zinc via Nitro-PAPS {2-(5-Nitro-2-pyridylazo)-5-(N-n-propyl-N-(3-sulfopropyl)amino)phenol, disodium salt, dihydrate $C_{17}H_{19}N_5Na_2O_6S.2H_2O$}method respectively.

Statistical analysis: All the statistical analysis will be carried out by using SPSS 29 (Statistical Package for Social Sciences) and MS Excel 2013. The quantitative variables were expressed as Mean and Standard deviation. The Z-test will be used for mean comparison between the groups for variables. The Pearson correlation analysis was used to find out the linear relationship among the variables. The $p < 0.05$ is considered as statistically significant and $P < 0.01$ – is considered as highly significant.

Observation and Results: Mean and standard deviations the clinical biochemical characteristics of the patients (cases) and controls of this study are summarized in Table 1 and Table 2. Among 40 cases with type 2 diabetes mellitus taking hypoglycemic drugs included in our study, 21 (52.5%) were the cases those who have been suffering from more than 2 years with type 2 diabetes mellitus taking hypoglycemic drugs in comparison with the cases those who have been suffering for less than 2 years taking hypoglycemic drugs (19 =47.5%).

The mean comparison of variables between cases and controls reveals that the fasting plasma glucose and total plasma homocysteine were significantly ($p< 0.001$) increased in cases as compared to controls. Similarly Serum Vitamin D, Serum Magnesium and Serum Zinc were significantly ($p< 0.001$) decreased in cases as compared to controls (Table no.: 1).

The mean comparison of variables between cases with DM taking hypoglycemic drugs for more than 2 years reveals that the total plasma homocysteine were significantly ($p< 0.001$) increased in cases with DM taking hypoglycemic drugs for more than 2 years as compared to with DM taking hypoglycemic drugs for less than 2 years. Similarly Serum Vitamin D, Serum Magnesium and Serum Zinc were significantly ($p< 0.001$) decreased in DM patients taking hypoglycemic drugs for more than 2 years as compared DM patients taking hypoglycemic drugs for less than 2 years (Table no.: 2).

Table No.: 1 showing mean comparison of variables between cases of DM and controls

Variable	Group	Number	Mean	Std. Deviation	Std. Error Mean	P-value
Total Plasma homocysteine	Case	40	21.44	2.762	0.44	p < 0.01
	Control	40	12.64	0.96	0.154	
Serum Vitamin D	Case	40	13.67	3.53	0.56	p < 0.01
	Control	40	29.55	3.22	0.51	
Fasting Blood Sugar	Case	40	178.83	39.14	6.19	p < 0.01
	Control	40	84.18	7.53	1.19	
Serum Zinc	Case	40	34.13	7.81	1.23	p < 0.01
	Control	40	89.38	14.09	2.22	
Serum Magnesium	Case	40	1.45	0.12	0.02	p < 0.01
	Control	40	2.03	0.14	0.02	

Table No.: 2 showing mean comparison of variables between cases of DM taking hypoglycemic drugs for more than 2 years and DM taking hypoglycemic drugs for less than 2 years

Parameters	Duration of DM	N	Mean	Std. Deviation	Std. Error Mean	P-value
Total Plasma Homocystein	<2years	21	23.6143	1.34212	0.29287	p < 0.01
	>2years	19	19.0368	1.70724	.39167	
Serum Vitamin D	<2years	21	16.5657	2.01270	.43921	p < 0.01
	>2years	19	10.4784	1.42397	.32668	
Fasting Blood Sugar	<2years	21	1.7829E2	40.18102	8.76822	p=0.92
	>2years	19	1.7942E2	39.05454	8.95973	
Serum Zinc	<2years	21	40.1429	4.33919	.94689	p < 0.01
	>2years	19	27.4737	4.71839	1.08247	
Serum Magnesium	<2years	21	1.5352	.03296	.00719	p < 0.01
	>2years	19	1.3521	.10158	.02330	
DM: diabetes Mellitus; < 2Years: less than 2 years; > 2Years: greater than 2 years						

Table 3 showing the Pearson correlation coefficient of variables among groups of DM

		Fasting Blood Sugar	Serum Magnesium	Serum Zinc	Serum Vitamin D
Total Plasma Homocystein	Pearson Correlation	0.323*	-0.325*	-0.550**	-0.73**
	Sig. (2-tailed)	0.042	0.041	0.000	0.000
	N	40	40	40	40
*. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed).					

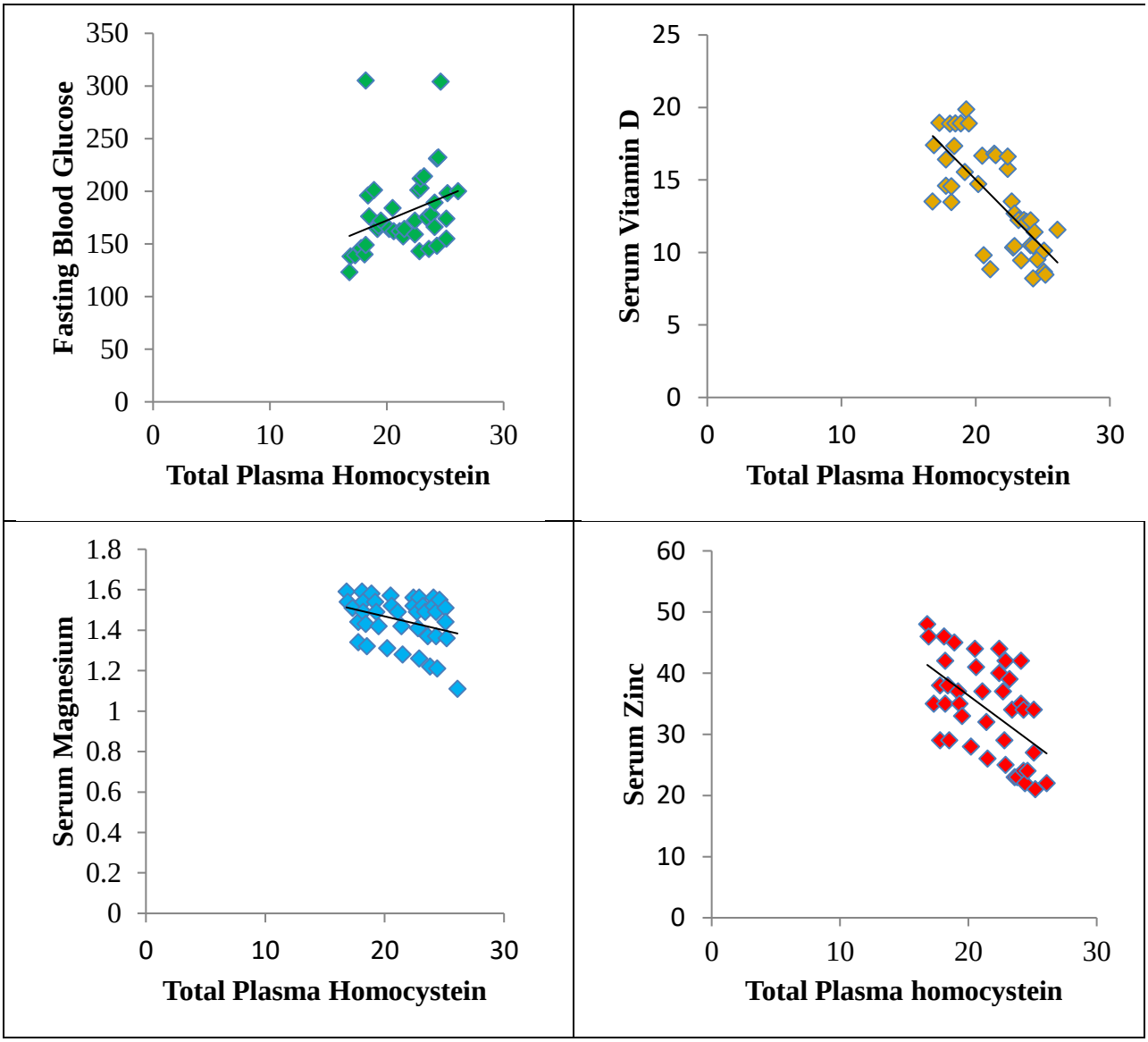

Figure no.: 1 showing the Pearson correlation coefficient of variables among DM subjects

The serum test of all the subjects reveals the correlation between homocysteine and glucose shows significant positive correlation while correlation between homocysteine with vitamin D, magnesium and Zinc that shows statistically significant negative correlation respectively ($p < 0.05$) (Figure no.: 1).

DISCUSSION: India has 77 million diagnosed diabetics, making it the second most affected country after China. In 2020, 700,000 Indians died from diabetes and related complications. India accounts for 17% of the global diabetic population, with numbers expected to reach 134 million by 2045 [18].

In the past three decades, diabetes-related deaths and Disability-adjusted Life Years (DALYs) in India have more than doubled. In 2019, the death rate was 19.64 per 100,000, and the DALYs rate was 919.02 per 100,000. Diabetes-related DALYs are linked to high risks for stroke, coronary artery disease, COPD, chronic kidney disease, and other conditions [19].

Metformin, the most prescribed oral hypoglycemic for type 2 diabetes, is used alone or with sulfonylureas. Unlike sulfonylureas, it is not bound to plasma proteins; neither metabolized, and is quickly eliminated by the kidneys. It lowers glucose by increasing glucose utilization in peripheral tissues and the intestine without stimulating insulin secretion, requiring insulin for its effect [20].

The association between diabetes and the increased risk for cardiovascular and Osteoporosis is well established. The long term usage of hypoglycemic drugs induces clinical complications and also alters the concentrations of trace elements, which may further lead to pathogenesis and progression of diabetes mellitus [21]. Thus the assessment of trace elements and magnesium along with the hypoglycemic drug impact must be added to the standardized care of diabetic patients which helps the clinicians to institute better treatment protocol for the benefit of the patient [22].

Among the 40 cases with type 2 diabetes mellitus taking hypoglycemic drugs included in our study, 21 (52.5%) were those who had been suffering from type 2 diabetes mellitus for more than 2 years and were taking hypoglycemic drugs, compared to 19 (47.5%). Fasting plasma hyperglycemia and total plasma homocysteine levels were considerably ($p<0.001$) higher in cases compared to controls. The mean comparison of variables between cases with DM taking hypoglycemic drugs for more than 2 years reveals that total plasma homocysteine were significantly ($p<0.001$) increased in cases with DM taking hypoglycemic drugs for more than 2 years compared to cases taking hypoglycemic drugs for less than 2 years. This is consistent with the findings of Platt DE et al. [23], and Ala OA et al. [24].

Serum Vitamin D, Magnesium, and Zinc levels fell considerably ($p<0.001$) in cases compared to controls. DM patients who took hypoglycemic drugs for more than 2 years had significantly reduced serum levels of Vitamin D, Magnesium, and Zinc ($p<0.001$). This is consistent with the findings of Gandhe MB et al. [25], and Diwan AG et al. [26]. All subjects' serum tests showed a substantial positive link between homocysteine and glucose, but a statistically significant negative correlation with vitamin D, magnesium, and zinc ($p<0.05$). This supports the findings of Yuan X [27], Kedari GSR [28], and Joe M [29]. Therefore, it is clear that the wide spread application of hypoglycemic drugs has increased the risk of osteoporosis, cardiovascular changes and mineral abnormalities and clinical and experimental evidence proves that hypoglycemic drugs plays a major role in changing many physiological conditions.

CONCLUSION: The study concludes that hypoglycemic drugs significantly affect patients on long-term treatment, necessitating prompt identification and resolution of associated changes. Serum markers can help gauge the onset and progression of these changes, providing insights into the clinical status of type 2 diabetes patients. The study aids clinicians in assessing the impact of biguanides on cardiovascular changes and osteoporosis based on diabetes duration, guiding better treatment protocols.

The timely screening of the parameters i.e. Vitamin D, Homocysteine, Zinc, and Magnesium, on the patients with prolonged intake hypoglycemic drug effects, shall be included in standard diabetic diagnostic protocol to improve patient outcomes.

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