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Original Article

Comparative Evaluation of Macro Minerals Status in Serum of Diabetes Mellitus Patients and Healthy Individuals

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ABSTRACT

Objective: To compare Vitamin B12, Vitamin D, Zinc, Calcium, Sodium, Potassium, Folate, Magnesium and Phosphate levels in Diabetes mellitus patients on oral hypoglycemics (OHG) with healthy population

Study Design: Comparative cross sectional study

Place and Duration of Study: Outdoor Patient Department, Pak Emirates Military Hospital Rawalpindi, Pakistan from Jan to Dec 2024

Methodology: Patients were selected into Group-1 (diabetic) and Group-2 (non-diabetic) according to inclusion and exclusion criteria using non-probability consecutive sampling method. A questionnaire drafted to collect additional information from patients about demographics and treatment being taken was used. Blood samples were collected on spot for Vitamin B12, Vitamin D, Zinc, Calcium, Sodium, Potassium, Folate, Magnesium, Phosphate and HbA1C levels. WHO classification of Diabetes mellitus was taken as case definition of Diabetes mellitus for Group-1 while persons who did not have the Diabetes mellitus (type-2) were enrolled as non-diabetic cases for Group-2. Comparison of the vitamin and mineral parameters between two groups was done using independent sample t-test and Mann Whitney U test for respective sample types at a *p*-value of <0.05 as significant.

Result: p values of Vitamin B12 and Zinc were significant between Group-1 and Group-2 while insignificant for other minerals and vitamins studied.

Conclusion: Patients with Diabetes mellitus on treatment have significantly low Vitamin B12 and Zinc values in comparison to healthy population

Keyword: Calcium, Diabetes mellitus, glycosylated hemoglobin, hypoglycemics, Magnesium, Potassium, Phosphate, Sodium, Vitamin B12, Vitamin D, Zinc

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INTRODUCTION

Diabetes mellitus is a group of disorders leading to persistently high blood glucose levels.[1] It results from either non-functioning pancreatic beta cells causing less / absent insulin production (Diabetes mellitus type 1) or increased peripheral insulin resistance (Diabetes mellitus type 2). Other forms of Diabetes mellitus such as gestational Diabetes mellitus and monogenic Diabetes mellitus have their own distinct pathophysiology. Persistent raised serum glucose values result in several micro and macro vascular complications ultimately ending in catastrophe of end organ damages. It is a chronic disease process though may be diagnosed with some acute complications as well such diabetic ketoacidosis.[2]

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Diabetes mellitus type 2 accounts for 90% cases of Diabetes mellitus. Initially considered to be a disease of the elderly, it effects the younger population as well. With sedentary lifestyle and diet at its root, its incidence is rapidly rising in the middle to lower income countries causing a significant financial burden. The current management of Diabetes mellitus revolves around dietary and lifestyle modification, oral hypoglycemics drugs (OHG) and / or insulin with an aim to keep the blood glucose within normal limits.[3]

Minerals, either macro or trace, play a vital part in functioning and maintenance of human body.[4] They are classified into macro-minerals including Calcium, Phosphorus, Magnesium, Sodium, Potassium, Chloride and sulfur and trace or micro-minerals including Zinc, Copper, Selenium and Manganese.[4] They play a vast array of roles in the body, including maintaining blood osmolality, bone mineralization,

cellular membrane action potentials and serving as coenzymes in various essential metabolic pathways.5 Vitamins and minerals interplay in functioning of body at cellular level forming coenzymes in many metabolic pathways which are essentials of life and its wellbeing.

Diabetes mellitus is associated with a number of micro and macro nutrient derangements. These imbalances may be cause of many complications which can be individually ascertained to a particular mineral or vitamin[8] such as, Vitamin B12 deficiency6 resulting from decreased absorption caused by metformin is a long known established fact.

This study was done with an aim to explore the presence of mineral derangements in diabetic population, comparison of Calcium, Phosphate, Magnesium, Sodium, Potassium, Zinc, Folate along-with Vitamin D and B12 in patients with Diabetes mellitus as compared to the healthy population. Previously no comprehensive study has compared the vitamin levels along with the macro and micro minerals, So, this study is aimed to open new avenues of understanding of Diabetes mellitus and its management. As it is a huge field so only relationship of variables in patients having Diabetes mellitus taking oral hypoglycemics (OHG) was studied.

METHODOLOGY

This cross-sectional comparative study was carried out in Outdoor Patient Department (OPD) of Pak Emirates Military Hospital (PEMH) Rawalpindi, Pakistan from January 2024 to December 2024 after ethical approval from Ethical Review Board (vide letter number A/28/ERC/140/24). Based on a confidence interval of 95%, power of test 80%, using the reported mean level of Vitamin B12 as 311±194.4 for diabetic group and 414± 223.5 for non-diabetic group 9, sample size of 138 (69 cases, 69 controls) was calculated by OpenEpi, Version 3, open source calculator[10]. Non-probability consecutive sampling technique was used, and only qualified doctors performed the data collection. All data collectors and authors attended a joint session prior to commencement of study for standardization and quality assurance in data collection.

Inclusion Criteria: Participants from both genders having age between 40 - 80 years coming to OPD as walk-in individuals, willing for being a part of this research after informed verbal consent were included. They were divided into two groups. Group-1 included participants diagnosed with Diabetes mellitus (type 2) as per WHO criteria11 and on oral hypoglycemics for past 1 year at least assessed through HbA1c levels and clinical investigation while, Group-2 participants were healthy individuals who did not have the Diabetes mellitus. (figure)

Exclusion Criteria: Individuals with chronic diarrhea and major illness requiring routine treatment assessed clinically, history of major abdominal surgery involving gut resection, taking supplements in past 6 months, recent history of acute illness in last six months requiring admission or intravenous therapy and impaired cognition were excluded. To control bias, patients on insulin therapy are also excluded to avoid confounding.

Data collection proforma containing demographic characteristics and basis information about the disease itself, including age, gender, smoking history, rural or urban living, duration of disease and treatment and number of drugs being taken for management was filled up by authors and data collectors in Outdoor patient settings from participants fulfilling the criteria.

20cc Venous blood samples of participants of both groups were collected on spot to measure serum Calcium, Phosphate, Magnesium, Sodium, Potassium, Zinc, Vitamin B12, total Vitamin D, serum Folate and HbA1c levels. Roche Cobas Pro c503 chemical and endocrine analyzer was used for measurements of results of above-mentioned minerals and vitamins less serum level of HbA1c, which was measured by Capillays 3 Octa system and cut off value for diagnosis was taken as >6.4% as per WHO case definition to confirm the cases being diabetic or non-diabetic.

The Statistical Package for Social Sciences Version 26 (SPSS Ver.26) was used for statistical calculations. The normality was checked for all quantitative variables using Shapiro Wilk Test which showed age, sodium and zinc as parametric while HbA1C, calcium, phosphate, magnesium, potassium, folate, Vitamin D and B12 as non-parametric variables along-with duration of diagnosis of Diabetes mellitus in Group-1. For categorical data, frequency and percentages were calculated. Mean ± standard deviation was used for continuous parametric while median with IQR (Inter Quartile Range) was used for non-parametric variables. Comparison of vitamin and mineral parameters values between diabetic and non-diabetic groups was done using independent sample t-test for parametric variables and Mann-Whitney U test for

non-parametric variables at a p-value of <0.05 taken as significant.

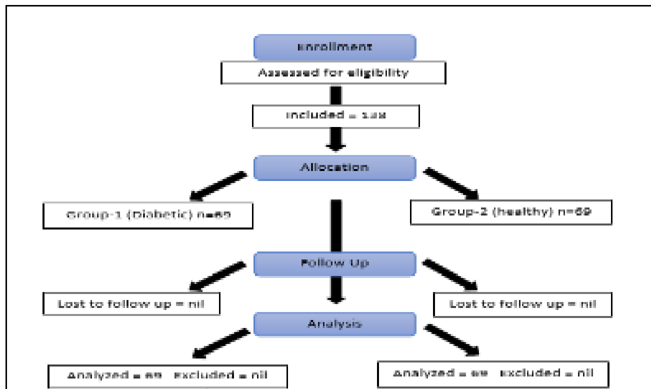

Figure : Patient flow chart (n=138)

RESULTS

A total of 138 patients were enrolled in the study with 69 patients in each group i.e Diabetic and Healthy Groups. There were 36(52.2%) males in Diabetic group while 33(47.80%) were female while normal patients' group had 45(65.2%) males and 24(34.80%) females. 103(74.6%) were found smokers while 35(25.40%) were non-smokers. The mean age of patients was 60.75±7.20 years. In Group-1, the median duration of Diabetes mellitus with IQR was 96.00(60.00 – 120.00) months and median value of HbA1c was 6.450 % (5.40 – 8.73). The mode number of hypoglycemics taken by diabetic patients was 3.00. The baseline features of sample population with demographic and clinical variables are shown in Table-I.

Table-I: Demographic Variables of Study Participants (n=138)

Variable	Group-1 (Diabetic) (n=69)	Group-2 (Non-diabetic) (n=69)	Total
Gender			
Male	36(26.1%)	45 (32.6%)	81 (58.7%)
Female	33 (23.9%)	24 (17.4%)	57 (41.3%)
Address			
Urban	32 (23.2%)	16 (11.6%)	48 (34.8%)
Rural	37 (26.8%)	53 (38.4%)	90 (65.2%)
Smoker			
No	52 (37.7%)	51 (37%)	103 (74.6%)
Yes	17 (12.3%)	18 (13%)	35 (25.4%)
Mean age of patients (years)		60.75±7.20	

Normality of all primary outcome variables was checked using Shapiro-Wilk Test. Only Serum Zinc and Serum Sodium levels were found to normally distributed while all other primary outcome variables were not normally distributed. On comparison of the vitamin level and macro mineral values in diabetic patients (Group-1) and non-diabetic population (Group-2), it was observed that the mean serum Zinc level (mmol/L) was 13.57±1.43 in diabetic and14.39±1.46 in non-diabetic groups which was significant (*p* = 0.001) amongst two study groups. Mean serum Sodium (mmol/L) was 139.34±3.30 in diabetic and 140.4±3.56 in the non-diabetic groups which showed insignificant (*p* = 0.069) differences for the study parameters. Only Vitamin B12 levels showed significant difference with *p*-value (<0.001) amongst two group. There were differences between serum levels of other study parameters amongst diabetic patients and healthy individuals, but no significance was found. These results have been summarized in the Table-II.

DISCUSSION

The results of this study showed that serum Zinc and Vitamin B12 levels were significantly lower in diabetic patients compared to healthy individuals (*p* = 0.001 for both), highlighting potential micronutrient deficiencies associated with either Diabetes mellitus, its treatment or both. These findings align with previous literature suggesting altered trace element homeostasis in diabetic states due to increased urinary loss, impaired absorption, or chronic inflammation. Our study showed similar results like Hasanato *et al* studied the relative alterations in copper, Zinc and selenium levels in diabetic and non-diabetic patients.[12] They found higher values of mentioned minerals in patients with Diabetes mellitus than the controls which were not having any Diabetes mellitus. These results for Zinc were different than this study which needs further data and research. However, the differences may have resulted from dietary variations in our population. Another macro-mineral that has not been studied in our research was sulfur. Ogunleye and Asaolu have reported that sulfur also had a negative association with Diabetes mellitus as compared to healthy individuals.[13]

Patients with type 2 Diabetes mellitus, who ultimately require insulin in the course of their disease, may also be affected by disturbed mineral levels, a phenomenon that has been studied by Ramaswamy *et al.* He has suggested that mineral alterations affect insulin sensitivity at higher HbA1c levels.[14] The deficiencies of copper, Zinc and selenium have even been implicated in the pathogenesis of Diabetes mellitus by Geir Bjørklund et al in their review.[15] A meta-analysis done by Li et al. advocated for vitamin and mineral supplementation in mothers

with gestational Diabetes mellitus because of improvement in glycemic control.[16]

The complications of Diabetes mellitus have also been associated with minerals. These linkages were shown by Sivaprasad *et al*.[17] for chronic kidney disease and Thomas et al.[18] for periodontitis in Diabetes mellitus patients. Moore et al. evaluated the effects of nutritional supplements on healing of feet ulcers in Diabetes mellitus patients. Although, no clear healing benefits were evident in literature review but there was clear improvement in morbidity and requirement of amputations.[19]

The authors of this study suggest that the treatment of Diabetes mellitus especially with oral hypoglycemics should also include supplementation with important minerals and vitamins on periodic basis if not routinely and further studies are required to establish this. This is supported by the work of Xia, J. *et al* who have studied comparative effects of supplementation of vitamins and minerals in managing Diabetes mellitus mellites type 2. They have suggested that compared to other micronutrients, supplements especially Chromium, Vitamin E, Vitamin K, Vanadium, and Niacin Supplements, are more effective in the management of T2DM.[20] Contrasting opinion suggests that mineral supplementation also poses additional risks of their toxicities and limited data is available for their safe values in patients with Diabetes mellitus highlighted for chromium in a study by Suksomboon *et al*.[21]

In light of these findings and supporting literature, it becomes evident that micronutrient disturbances particularly involving Zinc and Vitamin B12 are prevalent among patients with type 2 diabetes mellitus and may progress with disease duration. The implications of these deficiencies extend beyond glycemic control to potential complications and long-term outcomes. However, while micronutrient supplementation may offer therapeutic benefits, careful monitoring and individualized assessment are essential to avoid adverse effects or toxicities. Future research with larger sample sizes and longitudinal follow-up is warranted to establish evidence-based guidelines for routine screening and supplementation in diabetic care. Also, further studies are required to establish fact if these findings are because of Diabetes mellitus itself or due to its treatment.

CONCLUSION

This study found significantly lower serum Zinc and Vitamin B12 levels in diabetic patients compared to non-diabetics, with Zinc showing a significant decline over longer Diabetes mellitus duration. These findings suggest potential benefits of monitoring and supplementing key micronutrients, particularly Zinc and B12, in diabetic care. Further research is needed to guide clinical practice.

Table-II: Comparison of Mean Mineral Value In Group-1 versus Group-2 (n=138)

Variable(s)	Parameters	Group-1 (Diabetic) (n=69)	Group-2 (Healthy) (n=69)	*p*-value
Serum Sodium (mmo/L)	Mean ± S.D	139.34±3.30	140.4±3.56	0.069
Serum Zinc (μmol/L)		13.57±1.43	14.39±1.46	0.001
Serum Calcium (mmol/L)	Median (IQR)	2.33 (2.21 – 2.41)	2.34 (2.21 – 2.50)	0.167
Serum Phosphate (mmol/L)		1.18 (1.02 – 1.25)	1.14 (1.03 – 1.29)	0.856
Serum Magnesium(mmol/L)		0.95 (0.89 – 1.02)	0.95 (0.89 – 1.03)	0.428
Serum Potassium (mmo/L)		4.60 (4.00 – 5.00)	4.70 (4.15 – 5.00)	0.199
Serum Vitamin B12 (pmol/L)		158.00 (120.00 – 199.00)	254.00 (207.00 – 287.00)	<0.001
Serum Vitamin D (nmol/L)		55.67 (39.72 – 76.78)	56.45 (45.62 – 89.43)	0.195
Serum Folate (nmol/L)		29.00(20.50 – 37.37)	28.00 (19.00 – 38.50)	0.682

LIMITATIONS OF STUDY

First, it was conducted at a single center, which may limit the generalizability of the findings to broader populations with different dietary, genetic, or environmental factors. Second, patients with comorbid conditions other than Diabetes mellitus were excluded, which, while helping to reduce confounding, may not fully reflect real-world clinical scenarios where multiple comorbidities commonly coexist. Third, the analysis focused on a limited panel of vitamins and minerals, omitting potentially relevant micronutrients such as copper, selenium, chromium, and sulfur, which have been implicated in glycemic regulation and diabetic complications in other studies. Fourthly, this study could not differentiate if the results were related to Diabetes mellitus itself or to its treatment. Lastly, the cross-sectional nature of the study precludes causal inference and highlights the need for prospective and interventional studies to better understand these associations.

Conflict of Interest: None.

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Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

MA & FH: Data acquisition, data analysis, critical review, approval of the final version to be published.

RP & S: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

AR & MI: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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