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Assessment of Serum Vitamin B12 and Vitamin D Levels among COVID-19-Vaccinated Hypertensive and Diabetic Population: A Cross-Sectional Study

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ABSTRACT:

Introduction: Hypertension and type 2 diabetes mellitus (T2DM) are associated with altered micronutrient status and may influence metabolic responses following COVID-19 vaccination. This study compared serum vitamin B12 and vitamin D levels among COVID-19 vaccinated adults with hypertension, T2DM, and coexisting hypertension with T2DM.

Materials and Methods: This cross-sectional study included 240 adults who had received at least two doses of a COVID-19 vaccine. Participants were categorized into hypertension (n=86), T2DM (n=70), and hypertension with T2DM (n=84) groups. Fasting blood glucose (FBG), glycated haemoglobin (HbA1c), serum vitamin B12, and serum vitamin D concentrations were measured. Statistical analysis was performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA).

Results: The mean age was highest in Group 3 (60.43±10.27 yr), followed by Group 1 (56.87±14.72 yr) and Group 2 (54.96±11.10 yr). FBG and HbA1c differed significantly among the study groups (P<0.001). Serum vitamin B12 concentrations were 313.29±234.69 pg/mL, 325.39±193.28 pg/mL, and 448.48±297.75 pg/mL in Groups 1, 2, and 3, respectively. Corresponding vitamin D concentrations were 33.51±18.61 ng/mL, 34.92±16.51 ng/mL, and 49.63±27.86 ng/mL, with significant differences across the groups (P=0.001 for vitamin B12 and P<0.001 for vitamin D).

Conclusions: Patients with both hypertension and T2DM demonstrated significantly elevated vitamin B12 and vitamin D levels following COVID-19 vaccination. These

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findings suggest possible immune-metabolic interactions, supporting routine micronutrient monitoring in individuals with chronic metabolic disorders.

1. BACKGROUND

Hypertension and type 2 diabetes mellitus (T2DM) represent significant global health challenges, with India reporting a prevalence of approximately 29.8% for hypertension and 11.8% for T2DM among adults, substantially contributing to cardiovascular and metabolic complications [1]. These conditions are recognized as key comorbidities in COVID-19, elevating the risk of severe illness and death [2]. A large-scale Indian study involving 813 COVID-19 patients revealed that T2DM was linked to a 2.46-fold increased likelihood of severe disease and a 2.11-fold higher risk of mortality, whereas hypertension's impact was less pronounced after age adjustment [3]. According to the American Diabetes Association, patients with T2DM, particularly those with poor glycemic control (HbA1c >10%), experience worse COVID-19 outcomes due to weakened immune responses and heightened inflammation [4]. Cyanocobalamin, a synthetic vitamin B12, supports DNA synthesis, red blood cell production, and neurological health, serving as a coenzyme in converting methylmalonic-CoA to succinyl-CoA and homocysteine to methionine [5]. Its deficiency, often seen in T2DM patients using metformin, can hinder immune cell activity and increase inflammation through elevated homocysteine [6]. Similarly, Vitamin D, the main circulating form of vitamin D, modulates calcium balance and immune responses by activating the vitamin D receptor, promoting antimicrobial peptides like cathelicidin, and suppressing pro-inflammatory cytokines[7]. Low Vitamin D levels, frequently observed in hypertension and T2DM, are associated with insulin resistance and vascular issues [8]. Both nutrients are vital for immune regulation, and their deficiencies may worsen COVID-19 outcomes in patients with comorbidities. Following COVID-19 vaccination, immune activation may influence micronutrient metabolism. Vaccines like Covishield and Covaxin, approved in India, trigger strong immune responses, potentially increasing the demand for vitamins B12 and D [9]. Research suggests that low Vitamin D levels might reduce vaccine effectiveness, while B12 deficiency could impair antibody production due to its role in immune function [10]. In India, where 50–90% of the population is vitamin D deficient and T2DM patients often have low B12 levels, the post-vaccination nutrient status of individuals with hypertension, T2DM, or both remains poorly studied [11]. This study investigates serum Vitamin B12 and Vitamin D levels in vaccinated patients with hypertension, T2DM, or both conditions to evaluate their nutritional status in the context of COVID-19 comorbidities.

2. MATERIALS AND METHODS

2.1 Study design and participants

This cross-sectional study was conducted at the College of Paramedical Sciences, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India. Participants were consecutively recruited from the OPD of General Medicine, Teerthanker Mahaveer Hospital & Research Centre, Moradabad. The study protocol was approved by the Institutional Ethics Committee, Teerthanker Mahaveer University (Ref. No. PM/ETHICAL/COPS/2023/021), and written informed consent was obtained from all participants before enrolment. A total of 240 participants were included in the study, comprising 86 patients with hypertension (Group 1), 70 with type 2 diabetes mellitus (Group 2), and 84 patients with both hypertension and T2DM (Group 3).

2.2 Blood sample collection and laboratory analysis

Following an overnight fast of 8–12 h, venous blood samples were collected under aseptic conditions using appropriate vacutainer tubes. Approximately 2 mL of blood was collected in a serum separator (clot activator) tube for estimation of serum vitamin B12 and vitamin D. After clot formation, the samples were centrifuged at 3000 rpm for 10 min, and the separated serum was aliquoted for biochemical analysis. Approximately 2 mL of blood was collected in a sodium fluoride vacutainer for FBG estimation, while 2 mL was collected in an EDTA vacutainer for HbA1c analysis. FBG was measured using the glucose oxidase–peroxidase method on an Erba clinical chemistry analyser (Erba Mannheim, Germany). Serum vitamin B12 and vitamin D concentrations were determined using an automated enzyme-linked fluorescent assay (Mini VIDAS®, bioMérieux, France). HbA1c was estimated by ion-exchange high-performance liquid chromatography (D-10 Hemoglobin Testing System, Bio-Rad Laboratories, Hercules, CA, USA). All analyses were performed in an NABL-accredited laboratory following the manufacturers' calibration procedures and internal quality control protocols. Vitamin B12 deficiency was defined as serum concentrations <200 pg/mL, whereas vitamin D deficiency was defined as serum 25(OH)D concentrations <20 ng/mL.

2.3 Data collection

Demographic characteristics, clinical history, antihypertensive and antidiabetic medication use, COVID-19 vaccine type, time since the last vaccine dose were recorded using a structured case record form.

2.4 Statistical analysis

Statistical analysis was performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). The normality of continuous variables was assessed using the Shapiro–Wilk test. Continuous variables are presented as mean ± standard deviation. Comparisons among the three study groups were performed using the Kruskal Wallis test. Categorical variables were expressed as frequencies and percentages. A two-tailed P value <0.05 was considered statistically significant.

3. RESULTS

3.1 Age of Study Populations

The mean age was 56.87 ± 14.72 yr in Group 1, 54.96 ± 11.10 yr in Group 2, and 60.43 ± 10.27 yr in Group 3. Among females, the mean age was 57.35 ± 14.31 yr in Group 1, 58.65 ± 9.09 yr in Group 2, and 59.40 ± 9.50 yr in Group 3. Among males, the mean age was 56.40 ± 15.29 yr in Group 1, 53.15 ± 11.62 yr in Group 2, and 61.36 ± 10.95 yr in Group 3. The age distribution across the three study groups is presented in Table 1.

Table 1: Mean Age of Study Participants.

Group	Overall Age Mean SD (Year)	Females Age Mean SD (Year)	Males Age Mean SD (Year)
Group 1	56.87 ± 14.72	57.35 ± 14.31	56.40 ± 15.29
Group 2	54.96 ± 11.10	58.65 ± 9.09	53.15 ± 11.62
Group 3	60.43 ± 10.27	59.40 ± 9.50	61.36 ± 10.95

All value were mean and SD. Group 1: Hypertension (n=86); Group 2: Type 2 diabetes mellitus (n=70); Group 3: Hypertension with type 2 diabetes mellitus (n=84). SD: Standard deviation.

3.2 Gender Distribution in Study Populations

The study population comprised 106 females (44.17%) and 134 males (55.83%). Group 1 included 43 females (17.92%) and 43 males (17.92%), Group 2 included 23 females (9.58%) and 47 males (19.58%), and Group 3 included 40 females (16.67%) and 44 males (18.33%). The sex distribution of the study participants is presented in Table 2.

Table 2: Gender Distribution in Study Populations

Group	Females (n, %)	Males (n, %)
Overall	106 (44.17%)	134 (55.83%)
Group 1	43 (17.92%)	43 (17.92%)
Group 2	23 (9.58%)	47 (19.58%)
Group 3	40 (16.67%)	44 (18.33%)

All values were frequency and percentage. Group 1: Hypertension (n=86); Group 2: Type 2 diabetes mellitus (n=70); Group 3: Hypertension with type 2 diabetes mellitus (n=84). SD: Standard deviation.

3.3 Descriptive Analysis of Glycemic Profile and Vitamins level in Study Population

The descriptive statistics of FBG, HbA1c, serum vitamin B12, and vitamin D across the three study groups are presented in Table 3. Mean fasting blood glucose and HbA1c levels were higher in Groups 2 and 3 than in Group 1. Mean serum vitamin B12 and vitamin D concentrations were highest in Group 3.

Table 3. Glycemic Profile and Vitamins (FBS, HbA1c, vitamin B12, and vitamin D) among study groups

Parameter	Group 1 (Mean ± SD)	Group 2 (Mean ± SD)	Group 3 (Mean ± SD)
FBG (mg/dL)	96.93 ± 13.12	168.93 ± 44.93	167.25 ± 44.52
HbA1c (%)	5.21 ± 0.36	7.66 ± 1.42	7.44 ± 1.36
Vitamin B12 (pg/mL)	313.29 ± 234.69	325.39 ± 193.28	448.48 ± 297.75
Vitamin D (ng/mL)	33.51 ± 18.61	34.92 ± 16.51	49.63 ± 27.86

Values are presented as mean ± standard deviation (SD). Group 1: Hypertension (n=86); Group 2: Type 2 diabetes mellitus (n=70); Group 3: Hypertension with type 2 diabetes mellitus (n=84). FBG, fasting blood glucose; HbA1c, glycated haemoglobin.

3.4 Glycemic Profile Comparison in Hypertensive, Diabetic and Comorbid Patients

Comparison of FBG and HbA1c among the three study groups using the Kruskal Wallis test is presented in Table 4. Median FBG and HbA1c values differed significantly among the study groups (P<0.001).

Table 4: Glycemic Profile Comparison in Hypertensive, Diabetic and Comorbid Patients

Parameter	Group 1	Group 2	Group 3	χ^2 (df=2)	*P* value
FBG (mg/dL)	96.93 ± 13.12	168.93 ± 44.93	167.25 ± 44.52	149.95	<0.001
HbA1c (%)	5.25 (44.41)	7.35 (167.53)	7.30 (159.21)	161.68	<0.001

Values are presented as mean ± standard deviation (SD). Group 1: Hypertension (n=86); Group 2: Type 2 diabetes mellitus (n=70); Group 3: Hypertension with type 2 diabetes mellitus (n=84). Comparisons were performed using the Kruskal Wallis test. FBG, fasting blood glucose; HbA1c, glycated haemoglobin.

3.5 Comparison of serum vitamin B12 and vitamin D among the study groups

Serum vitamin B12 and vitamin D concentrations differed significantly among the three study groups (P<0.001). The highest median concentrations of both biomarkers were observed in Group 3. The comparison is presented in Table 5.

Table 5. Comparison of serum vitamin B12 and vitamin D among the study groups

Parameter	Group 1 (Mean ± SD)	Group 2 (Mean ± SD)	Group 3 (Mean ± SD)	χ^2 (df=2)	*P* value
Vitamin B12 (pg/mL)	313.29 ± 234.69	325.39 ± 193.28	448.48 ± 297.75	14.47	<0.001
Vitamin D (ng/mL)	33.51 ± 18.61	34.92 ± 16.51	49.63 ± 27.86	19.37	<0.001

Values are presented as mean ± standard deviation (SD). Group 1: Hypertension (n=86); Group 2: Type 2 diabetes mellitus (n=70); Group 3: Hypertension with type 2 diabetes mellitus (n=84). Comparisons were performed using the Kruskal Wallis test. Vitamin D was measured as serum 25-hydroxyvitamin D.

4. DISCUSSION

The present cross-sectional study evaluated glycaemic parameters and serum vitamin B12 and vitamin D concentrations among vaccinated adults with hypertension (Group 1), type 2 diabetes mellitus (Group 2), and coexisting hypertension with type 2 diabetes mellitus (Group 3). Significant differences were observed in fasting blood glucose, HbA1c, serum vitamin B12, and vitamin D concentrations across the study groups. Group 3 demonstrated the highest mean concentrations of both vitamin B12 and vitamin D as well as the highest mean age. The demographic profile of the study population showed a slight male predominance, with males constituting 55.83 per cent of the participants. Group 3 also had the highest mean age (60.43±10.27

yr), whereas Groups 1 and 2 had mean ages of 56.87±14.72 yr and 54.96±11.10 yr, respectively. Increasing age is associated with a higher prevalence of hypertension and diabetes and may also influence vitamin D status because of reduced cutaneous synthesis and age-related metabolic changes Chia et al. (2018)[12]. As expected, fasting blood glucose and HbA1c were significantly higher among participants with diabetes alone and those with coexisting hypertension and diabetes than among participants with hypertension alone ($P<0.001$). These findings are consistent with Antwi-Baffour et al. (2023), in which chronic hyperglycaemia results from impaired insulin secretion and insulin resistance[13]. The similarity in glycaemic status between Groups 2 and 3 suggests that the coexistence of hypertension did not substantially alter overall glycaemic control in the present study Sun et al. (2019)[14]. Serum vitamin B12 concentrations differed significantly among the three study groups, with the highest mean concentration observed in Group 3. Vitamin B12 deficiency has frequently been reported among patients with type 2 diabetes, particularly in those receiving long-term metformin therapy because of impaired intestinal absorption Ko et al. (2014)[15]**.** Therefore, the relatively higher vitamin B12 concentrations observed in Group 3 may reflect differences in nutritional status, clinical follow-up, or treatment practices that were not specifically evaluated in the present study rather than the coexistence of hypertension itself Sobczyńska-Malefora et al. (2023)[16]. These findings should therefore be interpreted cautiously and warrant confirmation in studies incorporating dietary assessment, medication history, and vitamin supplementation. Serum vitamin D concentrations also differed significantly across the study groups and were highest in Group 3. Vitamin D plays an important role in glucose homeostasis, immune regulation, and cardiovascular health. Barbalho et al. (2018) studies have reported an inverse association between low vitamin D status and metabolic disorders, including type 2 diabetes mellitus and metabolic syndrome[17]. The comparatively higher vitamin D concentrations observed in Group 3 may be related to differences in lifestyle, nutritional intake, clinical management, or supplementation, although these variables were not evaluated in the present study[18]. Although all participants had received COVID-19 vaccination, the present study was not designed to evaluate the effect of vaccination on serum vitamin B12 or vitamin D concentrations. Consequently, the observed differences should be interpreted in relation to the underlying metabolic conditions rather than attributed to vaccination. Longitudinal studies including pre- and post-vaccination assessments would be required to clarify any potential influence of COVID-19 vaccination on micronutrient status. The present study has certain limitations. Information regarding dietary intake, vitamin supplementation, duration of diabetes and hypertension, renal function, body mass index, and sunlight exposure was not collected, all of which may influence serum vitamin B12 and vitamin D concentrations. Furthermore, the cross-sectional assessment of biochemical parameters limits causal interpretation.

5. CONCLUSION

Significant differences in fasting blood glucose, HbA1c, serum vitamin B12, and vitamin D concentrations were observed among patients with hypertension, type 2 diabetes mellitus, and coexisting hypertension with diabetes. Participants with both conditions demonstrated the highest mean vitamin B12 and vitamin D concentrations. These findings highlight the importance of routine assessment of micronutrient status in individuals with chronic metabolic disorders and support further prospective studies to determine the clinical significance of these observations.

Declaration

- **Consent for Publication:** Not applicable.
- **Conflict of Interest:** The authors declare that they have no conflict of interest.
- **Informed Consent:** Written informed consent was obtained from all participants prior to their enrolment in the study.
- **Ethical Approval:** The study was approved by the Institutional Ethics Committee, College of Paramedical Sciences, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India (Ref. No. PM/ETHICAL/COPS/2023/021).
- **Data Availability:** The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.
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- **Author Contributions:** S.S. and C.K. conceptualized and designed the study. B.D. performed participant recruitment, data collection, and laboratory investigations. F.C., H.K., and U.Y. contributed to data management, statistical analysis, and interpretation of the findings. S.S. and B.D. drafted the manuscript. C.K. and H.K. critically revised the manuscript for important intellectual content. All authors reviewed, approved the final version of the manuscript, and agreed to be accountable for all aspects of the work.

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