

Vegetarian versus Non-Vegetarian Differences in Serum Vitamin D3, Vitamin B12, Thyroid Profile and Glycemic Status (HbA1c) in an Indian Population: A Cross-Sectional Analytical Study

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Abstract

Background: Dietary pattern is an important, modifiable determinant of micronutrient status and may plausibly influence Vitamin D, Vitamin B12 and glycaemic control. Indian data that simultaneously examine these biomarkers, alongside thyroid function, according to dietary habits remain limited. The objective is to compare serum Vitamin D3, Vitamin B12, thyroid profile (FT3, FT4, TSH) and HbA1c between vegetarian and non-vegetarian adults attending a single center in India, and to examine the demographic correlates of these biomarkers. **Material and Methods:** This retrospective cross-sectional study was carried out among 200 patients attending Government Medical College Hospital, Dungarpur [Shree Haridev Joshi Hospital] undergoing routine laboratory investigation, classified as vegetarian (n = 125) or non-vegetarian (n = 75) by self-reported dietary habit. Serum Vitamin D3, Vitamin B12, FT3, FT4, TSH and HbA1c were measured by standard laboratory methods. Between-group comparisons used Welch's independent-samples t-test with Cohen's d as the effect-size estimate; categorical variables (sex, Vitamin D deficiency, Vitamin B12 deficiency, glycaemic control) were compared using the chi-square test, and Pearson correlation was used to explore associations between age and biomarker concentrations. A two-sided p-value < 0.05 was considered statistically significant. **Results:** Vegetarians were, on average, older than non-vegetarians (50.9 ± 16.5 vs 45.0 ± 17.2 years; p = 0.018). Mean Vitamin D3 was comparable between groups (19.48 ± 10.67 vs 18.80 ± 9.08 ng/mL; p = 0.632), as was HbA1c (6.50 ± 2.14% vs 6.51 ± 1.85%; p = 0.969). Vitamin B12 was numerically higher among non-vegetarians (468.19 ± 496.98 vs 358.84 ± 399.17 pg/mL; p = 0.108, Cohen's d = -0.25), a small-to-moderate but statistically non-significant effect. Thyroid parameters (FT3, FT4, TSH) did not differ significantly between groups, although FT3 showed a borderline trend (p = 0.091). Vitamin D deficiency (<30 ng/mL) was strikingly common in both groups (88.5% overall; 88.0% vegetarian vs 89.3% non-vegetarian, p = 0.954), and Vitamin B12 deficiency (<200 pg/mL) was present in 35.0% of the cohort with no significant between-group difference (p = 0.146). Age correlated positively with Vitamin B12 (r = 0.220, p = 0.002) and HbA1c (r = 0.215, p = 0.002), but not with Vitamin D3 (r = 0.103, p = 0.147). **Conclusion:** Vegetarian and non-vegetarian adults in this cohort demonstrated comparable Vitamin D3, thyroid profile and HbA1c levels. Vitamin B12 concentrations trended higher in non-vegetarians without reaching conventional statistical significance, and the near-ubiquitous prevalence of Vitamin D deficiency across both dietary groups suggests that dietary pattern alone does not adequately explain the burden of hypovitaminosis D in this population. Age emerged as a relevant correlate of Vitamin B12 and glycaemic status and merits consideration as a covariate in future, larger studies.

Keywords: Vegetarian, Non-vegetarian, Vitamin D3, Vitamin B12, HbA1c, Thyroid function, Glycaemic status, India, Cross-sectional study.

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INTRODUCTION

Micronutrient status is shaped jointly by dietary intake, absorption, sunlight exposure, body composition and, increasingly, by lifestyle factors linked to urbanisation. Among the deficiencies most frequently encountered in Indian clinical practice, Vitamin D and Vitamin B12 deficiency stand out for their exceptionally high prevalence across age groups, geographic regions and socioeconomic strata.^[1-10] Vitamin B12 is synthesised exclusively by micro-organisms and reaches the human diet almost entirely through animal-source foods; consequently, individuals who follow lacto-vegetarian or strict vegetarian dietary patterns are considered biologically predisposed to lower Vitamin B12 intake and, over time, to biochemical deficiency [2,4,7]. Vitamin D, in contrast, is synthesised endogenously on cutaneous exposure to ultraviolet-B

radiation, so its status is influenced less by diet per se and more by sun exposure, skin pigmentation, adiposity, air pollution, sunscreen use and time spent indoors.^[1,10]

Both micronutrients have also been implicated in pathways

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relevant to glucose homeostasis. Vitamin D is hypothesised to influence insulin secretion and peripheral insulin sensitivity through effects on pancreatic beta-cell function and systemic inflammation,^[11-13] while Vitamin B12 deficiency has been linked to hyperhomocysteinaemia and, indirectly, to insulin resistance, particularly in populations with high rates of metformin use.^[3,14,15] Plant-predominant dietary patterns have themselves been associated with modest benefits for glycaemic control in some interventional literature,^[6] which makes the joint evaluation of these micronutrients alongside HbA1c, a validated index of average glycaemia over the preceding two to three months, of particular relevance in a population such as India where vegetarianism is common and diabetes prevalence continues to rise.

Large-scale and pooled analyses continue to document a very high burden of Vitamin D deficiency in India and globally. A pooled analysis of population-based studies estimated that roughly 16% of participants worldwide had serum 25-hydroxyvitamin D below 30 nmol/L, with a substantially higher proportion below the more commonly used clinical threshold of 30 ng/mL.^[4] Indian regional surveys, including a representative sample from the National Capital Region of Delhi, have similarly reported deficiency in a large proportion of adults irrespective of urban or rural residence.^[9] However, relatively few Indian studies have simultaneously examined Vitamin D3, Vitamin B12, thyroid function and glycaemic status in the same cohort stratified by dietary habit, which limits the ability to judge whether diet is a meaningful independent correlate of these biomarkers once other demographic factors, such as age, are taken into account.

Thyroid hormones (free triiodothyronine [FT3], free thyroxine [FT4] and thyroid-stimulating hormone [TSH]) were also available in the present dataset. While a direct causal link between vegetarian dietary pattern and thyroid function is not firmly established, plant-based diets can differ in iodine, selenium and goitrogen content compared with mixed diets, and thyroid status can itself influence vitamin metabolism and glycaemic control. We therefore included a descriptive, hypothesis-generating comparison of thyroid parameters between dietary groups as a secondary objective.

Given this background, the present study was designed to compare serum Vitamin D3, Vitamin B12, thyroid profile and HbA1c between vegetarian and non-vegetarian adults attending a single Indian centre, to quantify the prevalence of Vitamin D and Vitamin B12 deficiency in each group, and to explore the relationship of age with these biomarkers as a potential explanatory covariate.

MATERIALS AND METHODS

Study Design and Setting: This retrospective cross-sectional analytical study was carried out among 200 patients attending Government Medical College Hospital, Dungarpur [Shree Haridev Joshi Hospital in medicine OPD and Inpatient department between [March,2025] and [June,2026]. Written informed consent was obtained from

all participants prior to enrolment, in accordance with the Declaration of Helsinki.

Study Population and Sampling: A total of 200 patients were enrolled using a consecutive/convenience sampling approach over the study period. Participants were classified into two dietary groups on the basis of self-reported habitual dietary practice: vegetarian (n = 125), defined as consuming no meat, fish, poultry or eggs, and non-vegetarian (n = 75), defined as regularly consuming meat, fish, poultry and/or eggs. and had complete biochemical data for the study variables were included. Individuals with known chronic kidney disease, malabsorption syndromes, pregnancy, current use of Vitamin D or Vitamin B12 supplementation, or known thyroid disorder on treatment were excluded to minimise confounding, consistent with the eligibility criteria applied at the time of recruitment.

Study Variables: The following variables were recorded for each participant: age (years), sex, dietary group (vegetarian/non-vegetarian), serum Vitamin D3 (ng/mL), serum Vitamin B12 (pg/mL), free triiodothyronine (FT3, pg/mL), free thyroxine (FT4, ng/dL), thyroid-stimulating hormone (TSH, μ IU/mL) and glycated haemoglobin (HbA1c, %). Glycaemic status was further dichotomised as good control (HbA1c <7.0%) or poor control (HbA1c $\geq$ 7.0%), consistent with commonly applied clinical thresholds.^[14]

Biochemical Analysis: Venous blood samples were collected under aseptic precautions after an appropriate period of fasting where applicable. Serum Vitamin D3 (25-hydroxyvitamin D) and Vitamin B12 were estimated using chemiluminescent immunoassay (CLIA). Thyroid parameters (FT3, FT4, TSH) were similarly measured by CLIA. HbA1c was estimated using high-performance liquid chromatography (HPLC), traceable to the National Glycohemoglobin Standardization Program (NGSP). Vitamin D status was categorised using the widely used clinical cut-off of deficiency at serum 25-hydroxyvitamin D <30 ng/mL, and Vitamin B12 deficiency was defined as a concentration <200 pg/mL, consistent with commonly used laboratory reference ranges.^[1,12]

Statistical Analysis: Data were analysed using Python (pandas, SciPy) SPSS version XX, if applicable. Continuous variables are presented as mean $\pm$ standard deviation (SD) and were compared between vegetarian and non-vegetarian groups using Welch's independent-samples t-test, which does not assume equal variances between groups. Standardised effect sizes were calculated as Cohen's d. Categorical variables (sex distribution, Vitamin D deficiency, Vitamin B12 deficiency and glycaemic status category) are presented as frequencies and percentages and were compared using the chi-square test of independence. Pearson correlation coefficients were computed to examine the linear relationship of age with Vitamin D3, Vitamin B12 and HbA1c. A two-tailed p-value < 0.05 was considered statistically significant throughout.

RESULTS

Two hundred patients were included in the final analysis, of whom 125 (62.5%) followed a vegetarian dietary pattern and 75 (37.5%) followed a non-vegetarian pattern. The overall cohort had a mean age of 48.7 ± 16.9 years (range 13-85 years) and

comprised 113 women (56.5%) and 87 men (43.5%).

Baseline Characteristics: Vegetarian participants were significantly older than non-vegetarian participants

[Table 1]. The sex distribution did not differ significantly between the two dietary groups, although non-vegetarians included a slightly higher proportion of men.

Table 1: Baseline demographic characteristics by dietary group

Characteristic	Vegetarian (n = 125)	Non-vegetarian (n = 75)	p-value
Age (years), mean $\pm$ SD	50.92 $\pm$ 16.45	44.99 $\pm$ 17.20	0.018*
Sex, female, n (%)	76 (60.8)	37 (49.3)	0.151
Sex, male, n (%)	49 (39.2)	38 (50.7)	-

*Statistically significant ($p < 0.05$); Welch's t-test for age, chi-square test for sex distribution.

Comparison of Biochemical Parameters: Mean Vitamin D3 and HbA1c concentrations were similar between vegetarian and non-vegetarian participants [Table 2, Figure 1]. Vitamin B12 was numerically higher among non-vegetarians by approximately 110 pg/mL, corresponding to a small-to-moderate effect size (Cohen's $d = -0.25$), but the

difference did not reach conventional statistical significance ($p = 0.108$). None of the thyroid parameters (FT3, FT4, TSH) differed significantly between groups, although FT3 showed a borderline trend toward higher values among non-vegetarians ($p = 0.091$).

Table 2: Comparison of biochemical parameters between vegetarian and non-vegetarian participants

Variable	Vegetarian Mean $\pm$ SD	Non-vegetarian Mean $\pm$ SD	p-value	Cohen's d
Vitamin D3 (ng/mL)	19.48 $\pm$ 10.67	18.80 $\pm$ 9.08	0.632	0.07
Vitamin B12 (pg/mL)	358.84 $\pm$ 399.17	468.19 $\pm$ 496.98	0.108	-0.25
HbA1c (%)	6.50 $\pm$ 2.14	6.51 $\pm$ 1.85	0.969	0.00
FT3 (pg/mL)	1.66 $\pm$ 0.42	1.83 $\pm$ 0.81	0.091	-0.29
FT4 (ng/dL)	9.40 $\pm$ 2.50	9.76 $\pm$ 2.53	0.335	-0.14
TSH (μ IU/mL)	5.55 $\pm$ 15.11	3.98 $\pm$ 6.76	0.315	0.12

Welch's independent-samples t-test; Cohen's d reported as (Vegetarian - Non-vegetarian) / pooled SD.

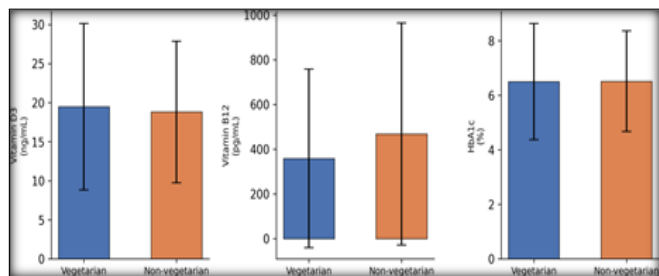


Figure 1: Mean ($\pm$ SD) serum Vitamin D3, Vitamin B12 and HbA1c by dietary group.

Prevalence of Micronutrient Deficiency: Vitamin D deficiency (<30 ng/mL) was present in the large majority of participants in both dietary groups, with an overall prevalence of 88.5% [Table 3, Figure 2]. There was no statistically significant difference in deficiency prevalence between vegetarians (88.0%) and non-vegetarians (89.3%; $p = 0.954$). Vitamin B12 deficiency (<200 pg/mL) was present in just over one-third of the cohort overall (35.0%), and was numerically more common among vegetarians (39.2%) than non-vegetarians (28.0%), although this difference also did not reach statistical significance ($p = 0.146$).

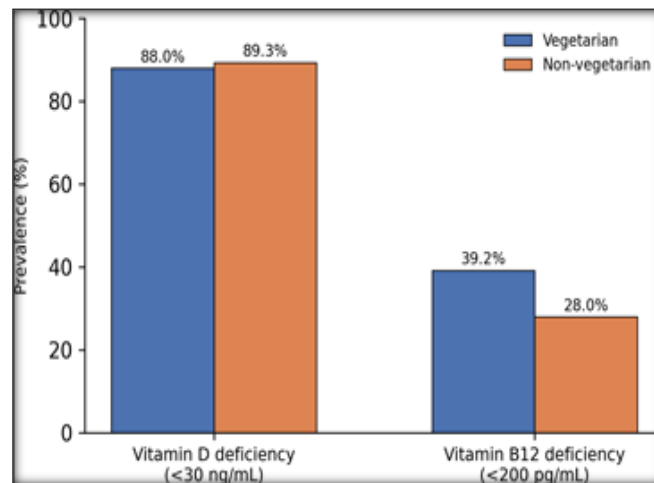


Figure 2: Prevalence of Vitamin D and Vitamin B12 deficiency by dietary group.

Glycaemic Status by Dietary Group: The distribution of glycaemic control categories did not differ significantly between dietary groups [Table 4]. Most participants in both groups had HbA1c in the good-control range ($<7.0\%$).

Table 3: Prevalence of Vitamin D and Vitamin B12 deficiency by dietary group

Deficiency status	Vegetarian n (%)	Non-vegetarian n (%)	Overall n (%)	p-value
Vitamin D deficiency (<30 ng/mL)	110 (88.0)	67 (89.3)	177 (88.5)	0.954
Vitamin B12 deficiency (<200 pg/mL)	49 (39.2)	21 (28.0)	70 (35.0)	0.146

Table 4: Distribution of glycaemic status by dietary group

Glycaemic status	Vegetarian n (%)	Non-vegetarian n (%)	p-value
Good control (HbA1c $<7.0\%$)	101 (80.8)	56 (74.7)	0.399
Poor control (HbA1c $\geq 7.0\%$)	24 (19.2)	19 (25.3)	-

Correlation of Age with Biomarkers: Age showed statistically significant, weak-to-moderate positive correlations with both Vitamin B12 and HbA1c, but not

with Vitamin D3 [Table 5], suggesting that older participants in this cohort tended to have higher Vitamin B12 and HbA1c values independent of dietary group.

Table 5: Pearson correlation of age with biochemical parameters

Variable pair	r	p-value
Age vs Vitamin D3	0.103	0.147
Age vs Vitamin B12	0.220	0.002*
Age vs HbA1c	0.215	0.002*
Vitamin D3 vs HbA1c	-0.004	0.955
Vitamin B12 vs HbA1c	0.013	0.858
Vitamin D3 vs Vitamin B12	0.086	0.225

*Statistically significant ($p < 0.05$).

Key Findings:

- Vitamin D3 and HbA1c levels were essentially identical between vegetarian and non-vegetarian participants.
- Vitamin B12 was numerically higher in non-vegetarians (small-to-moderate effect size) but the difference was not statistically significant.
- Vitamin D deficiency was present in almost 9 out of 10 participants overall, regardless of dietary group.
- Vegetarians in this cohort were significantly older than non-vegetarians, a potential confounder for age-related biomarkers.
- Age correlated positively with Vitamin B12 and HbA1c but not with Vitamin D3.
- Thyroid parameters (FT3, FT4, TSH) did not differ significantly by dietary group.

DISCUSSION

In this cross-sectional analysis of 200 Indian patients, vegetarian and non-vegetarian participants showed closely comparable Vitamin D3 and HbA1c levels, while Vitamin B12 concentrations trended higher among non-vegetarians without reaching statistical significance. These findings both extend and add to the existing Indian literature on diet-related micronutrient status.

The absence of a significant between-group difference in Vitamin D3 is consistent with the biological understanding that cutaneous synthesis, rather than diet, is the principal source of Vitamin D for most Indians, since very few naturally occurring dietary sources of Vitamin D exist and food fortification remains limited.^[1,10] The overall deficiency prevalence observed here (88.5%) is markedly higher than pooled global estimates of roughly 16-48% for varying thresholds,^[4] but is broadly in line with several recent Indian single-centre studies, including one from central India reporting deficiency in 87.5% of young apparently healthy adults with no association between deficiency and dietary pattern ($p = 0.164$) [reported in recent literature], and with regional surveys from the Delhi National Capital Region documenting a high burden of hypovitaminosis D across both urban and rural adults.^[9] Taken together, our data reinforce the view that indoor lifestyles, sunscreen use, atmospheric pollution, skin pigmentation and limited food fortification are more important determinants of Vitamin D status in the Indian

setting than dietary pattern alone.

The trend toward higher Vitamin B12 in non-vegetarians is biologically plausible, since Vitamin B12 is essentially absent from unfortified plant foods and vegetarians must rely on dairy products or fortified foods for adequate intake.^[2,4,7] The corresponding effect size in our cohort (Cohen's $d = -0.25$) indicates a small-to-moderate difference that a larger sample may confirm this difference as statistically significant; the present study may therefore have been underpowered to detect this difference given the observed variance in Vitamin B12 concentrations, which showed wide variability in both groups. Notably, Vitamin B12 deficiency was still present in over a quarter of non-vegetarians (28.0%) in our cohort, a finding that echoes previous reports describing Vitamin B12 deficiency as endemic in the Indian population irrespective of dietary habit, likely reflecting the additional contribution of low dietary diversity, *Helicobacter pylori*-related atrophic gastritis, and widespread metformin use among individuals with diabetes.^[8,11,12]

An unanticipated but noteworthy observation in this dataset was that vegetarian participants were, on average, six years older than non-vegetarian participants. This age difference may reflect a generational and cultural pattern common in parts of India, where strict vegetarianism is more frequently practised by older adults and by individuals from particular religious or regional backgrounds, while younger, more urbanised individuals may be more likely to consume mixed diets. Because age correlated significantly with both Vitamin B12 ($r = 0.220$, $p = 0.002$) and HbA1c ($r = 0.215$, $p = 0.002$) in our cohort, age represents a plausible confounder that should be adjusted for in future multivariable analyses; it may, in part, mask or attenuate a true dietary effect on Vitamin B12, since older individuals are also known to have reduced intestinal Vitamin B12 absorption due to age-related atrophic gastritis, irrespective of diet.^[12,15]

HbA1c and thyroid parameters showed no clinically meaningful differences between dietary groups. This is broadly consistent with systematic reviews suggesting that, while plant-predominant dietary patterns may confer modest benefits for glycaemic control in interventional settings, the magnitude of these effects is generally small and may not be detectable in a cross-sectional observational comparison of habitual vegetarians versus non-vegetarians drawn from a general hospital population with heterogeneous disease status.^[6,13,14] The borderline trend toward higher FT3 among non-vegetarians ($p = 0.091$) is difficult to interpret given the absence of dietary

iodine or selenium intake data in this study, and should be regarded as hypothesis-generating rather than confirmatory; differences in thyroid hormone concentrations can also be influenced by non-dietary factors such as underlying thyroid disease prevalence, which was not systematically assessed here.

Overall, these findings suggest that, at least in this cohort, dietary habits alone are not dominant determinants of Vitamin D status, thyroid function or glycaemic control, while its association with Vitamin B12 status appears real but modest and is likely modulated by age and other non-dietary factors. This has practical implications: clinicians should not assume that non-vegetarian status is protective against Vitamin D deficiency, nor that vegetarian status alone reliably predicts clinically significant Vitamin B12 deficiency without biochemical confirmation, particularly in older adults.

Strengths and Limitations

Strengths

- Simultaneous evaluation of Vitamin D3, Vitamin B12, a full thyroid profile (FT3, FT4, TSH) and HbA1c in the same cohort, which is relatively uncommon in the existing Indian literature.
- Comparative statistical analysis using Welch's t-test, appropriate for groups with unequal variances and unequal sample sizes, supplemented by standardised effect sizes (Cohen's d).
- Exploration of age as a potential confounder through correlation analysis, adding interpretive depth beyond a simple two-group comparison.
- Clearly defined deficiency thresholds applied consistently across both dietary groups.

Limitations:

- The cross-sectional design precludes causal inference regarding the relationship between diet and biomarker status.
- This was a single-centre, hospital-based sample, which may not be representative of the general population and may be enriched for individuals with underlying illness.
- Dietary classification was based on a broad self-reported category (vegetarian/non-vegetarian) without quantification of nutrient intake, frequency of animal-food consumption, or use of dairy and eggs within the vegetarian group.
- Sunlight exposure, body mass index, physical activity, skin pigmentation and use of Vitamin D or calcium supplementation were not captured and could not be adjusted for.
- The unequal group sizes (125 vegetarian vs 75 non-vegetarian) and wide variance in Vitamin B12 concentrations may have limited statistical power to detect a true between-group difference.
- Thyroid autoantibody status and known thyroid disease history were not available, limiting interpretation of the FT3, FT4 and TSH comparisons.

CONCLUSION

In this cross-sectional study of 200 Indian adults, vegetarian

and non-vegetarian participants exhibited similar Vitamin D3, thyroid profile and HbA1c levels. Vitamin B12 concentrations were numerically higher among non-vegetarians, with a small-to-moderate effect size that did not reach statistical significance, and Vitamin B12 deficiency remained common even among non-vegetarians. The very high prevalence of Vitamin D deficiency across both dietary groups (88.5%) indicates that dietary habit alone is insufficient to explain the burden of hypovitaminosis D in this population, and that non-dietary factors such as sunlight exposure and lifestyle should be prioritized in preventive strategies. The significant age difference between dietary groups, together with the positive correlation of age with Vitamin B12 and HbA1c, highlights the need for age-adjusted, adequately powered, multicentric studies to clarify the independent contribution of dietary pattern to micronutrient and glycaemic status in Indian adults.

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Conflicts of interest

There are no conflicts of interest.

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