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RESEARCH ARTICLE

Impact of Vitamin D Deficiency on Cardio Metabolic Ageing in Middle Aged Adults in India: A Comparative Analysis

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Abstract: Advancing age is accompanied by a gradual deterioration in physiological function, resulting in an increased risk of chronic diseases and early mortality. Growing evidence suggests that inadequate vitamin D status may be associated with unfavourable metabolic and cardiovascular alterations. Nevertheless, information regarding the association of vitamin D status with integrated cardio-metabolic biomarkers of ageing in middle-aged adults is currently limited. Study Objective: To investigate whether cardio-metabolic biomarkers of biological ageing differ according to vitamin D status in middle-aged adults. This cross-sectional investigation included 174 middle-aged participants between 45 and 65 years of age. Cardio-metabolic ageing was evaluated using key cardiovascular indices, comprising resting heart rate, blood pressure, and heart rate variability measurements together with metabolic indicators such as HbA1c, body fat percentage, lipid profile, and waist-hip ratio. Serum vitamin D concentration was determined to classify participants according to vitamin D status. A Mann–Whitney U test was applied to evaluate between-group differences according to vitamin D status. Individuals with deficient vitamin D status exhibited greater waist-hip ratio, total cholesterol, and low density lipoprotein than adequate vitamin D status ($p<0.001$). Heart rate variability analysis further revealed impaired autonomic regulation among participants exhibiting lower circulating vitamin D concentrations. Overall, the findings support that inadequate levels of vitamin D are connected to an unfavourable cardiometabolic profile in middle-aged adults and may contribute to the early development of physiological changes related to ageing.

Keywords: Vitamin D Status, Glycosylated Haemoglobin, Biological Ageing, Waist-Hip Ratio, Body Fat Percentage

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Introduction

Ageing is a multifactorial biological process involving gradual deterioration of physiological integrity, resulting in a progressive decline in functional capacity and a greater likelihood of developing chronic disorders and an increased risk of early death. As individuals age, structural and functional alterations affect several organ systems, such as the autonomic

nervous, respiratory, cardiovascular, and renal systems, ultimately compromising overall physiological performance. This biological process is accompanied by age-related changes, including arterial stiffening, reduced pulmonary function, osteoporosis, and various degenerative conditions.

The progression of biological ageing reflects the combined influence of environmental, behavioural, and genetic factors, such as diet, physical activity, sleep quality, lifestyle choices, and inherited characteristics. Accordingly, biological age is considered to provide a more accurate reflection of overall health than chronological age. Biological ageing was evaluated using a panel of cardiovascular and metabolic biomarkers. Cardiovascular assessment consisted of Resting Heart Rate (RHR), blood pressure, and Heart Rate Variability (HRV), while the metabolic evaluation included Body Mass Index (BMI), serum lipid measurements, glycated haemoglobin (HbA1c), body fat percentage, and Waist-Hip Ratio (WHR) [1].

Essential micronutrients play a key role in healthy ageing, and deficiencies in these nutrients are increasingly recognized as modifiable risk factors for biological ageing. Growing evidence suggests that inadequate intake of essential micronutrients, particularly vitamin D, may adversely influence multiple physiological systems and contribute to age-related functional decline [2].

A large proportion of the world's population is affected by inadequate vitamin D status, making it one of the most widespread nutritional deficiencies [3]. In India, vitamin D deficiency represents a substantial public health challenge because of its widespread occurrence, whereas deficiency is prevalent across different age groups despite abundant sunlight. This paradox has been attributed to a combination of inadequate dietary intake, limited cutaneous synthesis resulting from reduced sunlight exposure, predominantly indoor lifestyles, and insufficient physical activity [4].

Although vitamin D is primarily recognized for its role in skeletal homeostasis, it also acts as a pleiotropic hormone involved in the regulation of numerous physiological processes. It contributes to the maintenance of cardiovascular regulation, glucose utilization, immune activity, and cellular equilibrium [5, 6]. Evidence from both experimental investigations and clinical studies suggests that vitamin D plays a role in regulating the renin–angiotensin system and inflammatory signalling pathways, supports endothelial function, regulates cellular proliferation, and contributes to glucose homeostasis. By acting through these pathways, vitamin D may influence cardiovascular and metabolic health and may be associated with the risk of hypertension, metabolic disorders, infectious diseases and autoimmune conditions, thereby increasing the overall burden of chronic illnesses [7].

Despite considerable research examining vitamin D status in relation to cardio-metabolic health, several important questions remain unresolved; previous studies have predominantly examined isolated cardiovascular or metabolic outcomes, older adults, or disease-specific populations. Although vitamin D has been extensively investigated, studies integrating multiple cardio-metabolic biomarkers of biological ageing in healthy middle-aged adults remain scarce. Evaluating these biomarkers during middle age may enable the identification of subclinical physiological alterations that precede the development of overt chronic disease. Additionally, despite the widespread distribution of vitamin D deficiency and increasing cardiometabolic risk in India, evidence from Indian middle-aged populations remains limited.

This study compared cardio-metabolic biomarkers of biological ageing between healthy middle-aged Indian adults with deficient and sufficient serum vitamin D concentrations. In this context, cardio-metabolic ageing refers to the cross-sectional assessment of metabolic and autonomic biomarkers that reflect early physiological changes rather than a direct measure of biological ageing. By investigating the association between vitamin D status and these biomarkers, the study sought to improve understanding of early age-related cardio-metabolic alterations and provide a foundation for future research aimed at promoting healthy ageing in the Indian population.

Materials and Methods

Study Participants

Individuals between 45 and 65 years of age who attended the outpatient health examinations at KS Hegde Charitable Hospital, Mangalore, Karnataka, were recruited for this cross-sectional study. Serum vitamin D concentration served as the basis for participant classification. Individuals with serum vitamin D levels below 20 ng/mL were allocated to the deficiency group, whereas those with concentrations ranging from 30 to 100 ng/mL comprised the sufficiency group, following the diagnostic thresholds proposed by [8]. Adults with serum vitamin D concentrations within the 20 to 29.9 ng/mL interval,

indicative of vitamin D insufficiency, were not included in the analysis to ensure clear distinction between the study groups. Participants with a history of diabetes mellitus, hypertension, chronic illnesses, or current vitamin D supplementation were also excluded to minimize potential confounding.

During the recruitment period (January 2021 to March 2022), 190 individuals underwent eligibility screening for inclusion in the study. Sixteen individuals were excluded due to comorbid conditions and artefacts in HRV recordings, with 174 participants ultimately fulfilling the inclusion criteria and were subsequently analyzed. Based on serum vitamin D concentrations, the study cohort was stratified into two comparable groups (n = 87 each) according to their serum vitamin D status, representing deficient and sufficient categories. Consecutive sampling was employed, and all eligible individuals who fulfilled the eligibility requirements and consented to participate were recruited in this research.

Sample Size

The calculated total sample was 174 participants (87 in each group). Sample size calculations were performed using n-Master software. The minimum number of participants required was estimated by incorporating an effect size of 0.425, a type-I error probability of 5% ($\alpha = 0.05$), and statistical power of 80%.

Experimental Procedure

Before initiating the study, approval was obtained from the Institutional Ethics Committee (IEC) of Nitte University under Approval No. NU/CEC/2021/112. All eligible participants were briefed about the purpose and methodology of the investigation, following which written informed consent was secured from every participant before any study-related procedures were undertaken. After obtaining a detailed medical history, participants underwent a comprehensive anthropometric assessment. Measurements of height, skinfold thickness, waist circumference, weight, and hip circumference were obtained using anthropometric protocols. These measurements were subsequently used to determine Body Mass Index (BMI), waist-hip ratio (WHR), and percentage body fat were subsequently calculated from the anthropometric measurements. Percentage body fat was derived from body density using Siri's equation, with body density estimated from skinfold measurements [9]. Cardiovascular evaluation was then performed and included assessment of Heart Rate Variability (HRV), resting heart rate, and blood pressure.

Lead II ECG was recorded for 5 minutes in the supine position under normal spontaneous breathing, using a sampling rate of 1000 Hz for accurate R-wave detection. Participants remained supine throughout the measurement period, and data were recorded using a PowerLab acquisition system with LabChart Pro software. HRV parameters analyzed included (TP) Total Power, (LF) Low Frequency, (HF) High Frequency, and the LF/HF ratio.

HF (0.15 to 0.40 Hz) and LF (0.04 to 0.15 Hz) are generally considered to represent sympathetic regulation of cardiac function. Additionally, the ratio of LF/HF was calculated to provide an estimate of sympathovagal balance regulating the cardiac autonomic [10].

To assess vitamin D status and metabolic parameters, venous blood (4 mL) was withdrawn from the median cubital vein following standard aseptic procedures. After collection, the blood samples were processed according to the manufacturer's protocol to obtain serum by centrifugation. Serum vitamin D concentrations were subsequently determined using a commercial assay kit sourced from Krishgen Biosystems (India).

Glycaemic status was assessed by measuring HbA1c using an automated analyser. Serum lipid analysis was performed with a semi-automated biochemical analyser sourced from Agappe Diagnostics (Switzerland) to determine (TC) total cholesterol, (TG) triglycerides, and (HDL) high-density lipoprotein. (LDL) Low-density lipoprotein concentrations were obtained by calculation according to the Friedewald equation rather than by direct measurement.

Statistical Analysis

All statistical analyses were conducted using IBM SPSS Statistics version 25.0. Continuous variables were summarized as mean ± Standard Deviation (SD). The distribution of the data was examined using the Shapiro–Wilk test to assess normality before further analysis. Since normality assumptions were not satisfied for several variables, comparisons between the two study groups were analysed using the Mann–Whitney U test because the data did not satisfy the assumptions of normality. All statistical tests were two-tailed, and statistical significance was defined as a p-value of <0.05.

Results

Table 1 summarizes the metabolic ageing parameters according to vitamin D status. Comparison between the groups showed that individuals with lower vitamin D concentrations had a significantly greater WHR than their vitamin D-sufficient counterparts ($p<0.001$) (Fig. 1).

In contrast, body fat percentage and HbA1c remained similar across both study groups, and the observed differences were not statistically significant. Evaluation of serum lipid parameters indicated a more atherogenic lipid profile in individuals with lower vitamin D concentrations. When compared with participants with sufficient vitamin D status, these individuals had significantly greater TC and LDL concentrations, accompanied by increased TC/HDL, TG/HDL, and ratio of LDL/HDL ($p<0.001$). In contrast, HDL levels were significantly lower among participants with deficient vitamin D status ($p<0.001$).

Although other lipid parameters differed significantly, TG concentrations remained similar across both study groups, with no statistically significant between-group difference.

Cardiovascular parameters stratified by vitamin D status are summarized in Table 2. (DBP) Diastolic blood pressure and parameters of HRV, namely LF (absolute-ab), HF (absolute-ab), LF and HF (normalized units - nu), and the ratio of LF/HF, were identified between the study groups, with higher values observed among participants with vitamin D deficiency. SBP, RHR, and TP did not show significant difference between the vitamin D deficient and sufficient vitamin D groups.

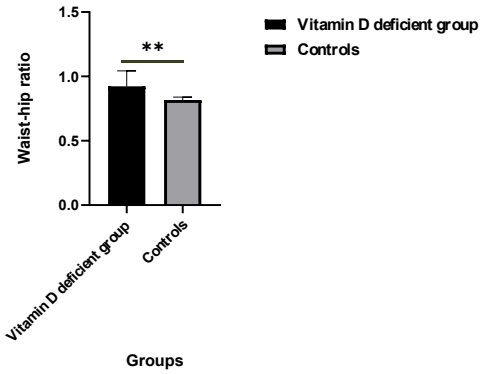

Fig. 1: Comparison of WHR between individuals with vitamin D deficiency and with sufficient vitamin D levels

Table 1: Differences in metabolic biomarkers of ageing between participants according to vitamin D status

S/L	Metabolic ageing biomarkers	Vitamin D Sufficient	Vitamin D Deficient	p value
1	Age (Years)	52.11±5.08	53.45±6.02	0.24
2	BMI (kg/m^2)	25.33±3.27	24.85±3.86	0.216
3	WHR	0.80±0.07	0.87±0.08	< 0.001*
4	Body fat %	23.55±3.12	24.46±2.55	0.902
5	HbA1c (%)	5.29±0.59	5.36±0.57	0.453
6	TC (mg/dl)	191.66±14.82	224.24±15.11	< 0.001*
7	TG (mg/dl)	141.59±60.46	153.17±58.47	0.077
8	LDL (mg/dl)	113.18±29.23	121.44±32.09	0.011*
9	HDL (mg/dl)	35.82±10.39	30.25±15.70	< 0.001*
10	LDL/HDL	3.05±1.18	3.57±1.19	0.01*
11	TC/HDL	4.18±0.13	4.62±0.12	< 0.001*
12	TG/HDL	2.68±1.59	4.29±2.33	< 0.001*

Statistical comparisons were conducted using the Mann–Whitney U test. Data are presented as mean ± standard deviation (SD). $p<0.05$ - statistically significant. TC, total cholesterol; WHR, waist-hip ratio; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglycerides; HbA1c, glycosylated haemoglobin.

Table 2: Differences in cardiovascular ageing biomarkers between participants with sufficient and deficient vitamin D levels

S/L	Cardiovascular ageing biomarkers	Vitamin D Sufficient	Vitamin D Deficient	p value
1	SBP (mmHg)	119.77±9.8	120.77±1.13	0.722
2	DBP (mmHg)	80.67±6.3	83.56±0.71	0.016*
3	RHR (bpm)	80.34±3.8	81.40±0.39	0.084
4	TP (ms^2)	4632.3±1100.4	4485.8±513.9	0.463
5	HF (ab) (ms^2)	892.7±180.9	1106.8±169.2	0.048*
6	LF (ab) (ms^2)	1134±175.3	995.6±249.2	0.034*
7	HF (nu)	38.41±2.28	33.72±1.89	0.041*
8	LF (nu)	46.2±2.42	53.47±2.36	0.014*
9	LF/HF ratio	0.63±0.47	1.33±0.56	< 0.001*

Statistical comparisons between groups using Mann–Whitney U test. Data are presented as mean ± Standard Deviation (SD). *p*-value <0.05 - statistically significant. DBP, diastolic blood pressure; SBP, systolic blood pressure; RHR, resting heart rate; LF/HF, low-frequency to high-frequency ratio; TP, total power; HF, high-frequency power; LF, low-frequency power

Discussion

Apart from its established role in mineral metabolism, vitamin D modulates several biological pathways, including inflammatory responses, oxidative stress, and metabolic functions. Cardio-metabolic ageing indices are commonly used as surrogate markers to assess age-related physiological changes rather than direct measures of biological ageing [11]. This investigation assessed whether selected metabolic and cardiovascular biomarkers differed according to vitamin D status among the study participants.

The present study identified notable differences in several metabolic and cardiovascular parameters between the groups, providing evidence linking vitamin D deficiency to an unfavourable cardiometabolic profile. Among the anthropometric variables evaluated, WHR was significantly elevated in individuals with vitamin D deficiency, suggesting greater central adiposity. The present result is similar to the research done by [12], which demonstrated a positive link between deficiency of vitamin D and abdominal obesity, a major contributor to cardiovascular risk. An elevated WHR, reflecting central adiposity has been widely recognized as a major contributor to metabolic and cardiovascular diseases. In this study, individuals with deficiency of vitamin D exhibited a significantly higher WHR, indicating greater abdominal fat accumulation. This finding suggests that diminished vitamin D status may be associated with increased susceptibility to obesity-related metabolic abnormalities. Moreover, Excess accumulation of abdominal adipose tissue is recognized as an important contributor to cardiovascular disease, insulin resistance, and hypertension, all of which may interact with systemic vitamin D levels [13].

Vitamin D Receptors (VDR) are expressed in adipose tissue, suggesting that vitamin D contributes to the regulation of adipose tissue physiology [13]. Although these biological mechanisms exist, neither BMI nor body fat percentage differed significantly according to vitamin D status.

Metabolic assessment included the measurement of HbA1c and various lipid profile parameters. Participants with vitamin D deficiency demonstrated a less favourable lipid profile, characterized by significantly greater TG, TC, TC/HDL, and LDL/HDL ratio, along with lower HDL levels, indicating the presence of dyslipidaemia. Comparable results were documented by [12], who proposed that insufficient vitamin D may reduce intestinal calcium absorption, thus stimulating lipolysis in adipose tissue. The resulting increase in free fatty acid mobilization may enhance hepatic triglyceride synthesis, contributing to higher serum triglyceride levels. Elevated levels of triglycerides can lead to the creation of triglyceride-enriched particles of HDL, making them more prone to degradation via hepatic lipase-mediated hydrolysis, followed by rapid removal from the bloodstream, thereby contributing to lower levels of HDL [14]. Moreover, participants with vitamin D deficiency in this current study exhibited a significantly greater ratio of TG/HDL, consistent with previous observations suggesting that diminished vitamin D status is associated with unfavourable lipid ratio profiles [15]. Furthermore, reduced vitamin D concentrations are accompanied by processes that may promote the development of atherosclerosis [16].

Analysis of the lipid profile showed that TC and LDL-C concentrations were higher in the deficient group than in participants with sufficient vitamin concentrations. This association between insufficient status of vitamin D and elevated cholesterol levels

may be attributed, at least in part, to alterations in LDL receptor activity, which could reduce hepatic LDL clearance, leading to decreased cellular uptake of cholesterol and consequently elevated circulating levels. Additionally, as cholesterol is an essential substrate in the cutaneous synthesis of vitamin D, alterations in this common metabolic pathway may affect both cholesterol homeostasis and serum vitamin D levels [14].

Maintenance of calcium homeostasis represents one of the primary biological functions of vitamin D; by promoting the intestinal absorption of calcium, this mechanism may indirectly limit fatty acid absorption, thereby contributing to reduced serum cholesterol levels [17]. Additionally, reduced levels of HDL were seen in vitamin D-deficient individuals, which aligns with earlier observations supporting a link between vitamin D insufficiency and unfavourable HDL profiles, suggesting that raised TG concentrations favour the formation of triglyceride-enriched particles of HDL, which are more readily hydrolysed and removed from the circulation, ultimately leading to a decrease in HDL concentrations [14].

Although glycosylated hemoglobin is considered a sensitive marker of metabolic ageing, no statistically significant disparity was identified across the study groups. A likely reason for this finding is the exclusion of individuals with diabetes, leading to relatively homogeneous glycaemic profiles across the study participants.

The present investigation assessed cardiovascular ageing through measurements of RHR, HRV and blood pressure. However, HRV indices showed no significant difference between the vitamin D groups. RHR and blood pressure remained comparable between the groups.

Investigation of HRV parameters revealed significant differences mainly in the normalized HF and LF components, as well as the LF/HF ratio (Figure 2), suggesting a shift in autonomic balance in vitamin D-deficient individuals. However, absolute HRV measures showed limited differences between groups. Therefore, these findings should be interpreted cautiously, as normalized HRV indices are influenced by total power and may not directly reflect absolute autonomic activity. Previous studies, including [18], have reported similar alterations in HRV parameters in individuals with low vitamin D levels, indicating potential changes in autonomic regulation. Ageing itself is also associated with reduced parasympathetic activity and relative sympathetic predominance [19].

The lack of significant differences in SBP, RHR, and Body fat percent between the groups may indicate that these parameters are influenced by multiple determinants beyond vitamin D status. Factors such as habitual physical exercise and dietary habits, which were not evaluated in this study, may provide a plausible explanation for these observations. Furthermore, the study population consisted of relatively healthy, middle-aged adults without advanced metabolic disease; in such cohorts, variations in vitamin D status may not be large enough to produce measurable changes in glycemic or cardiovascular parameters.

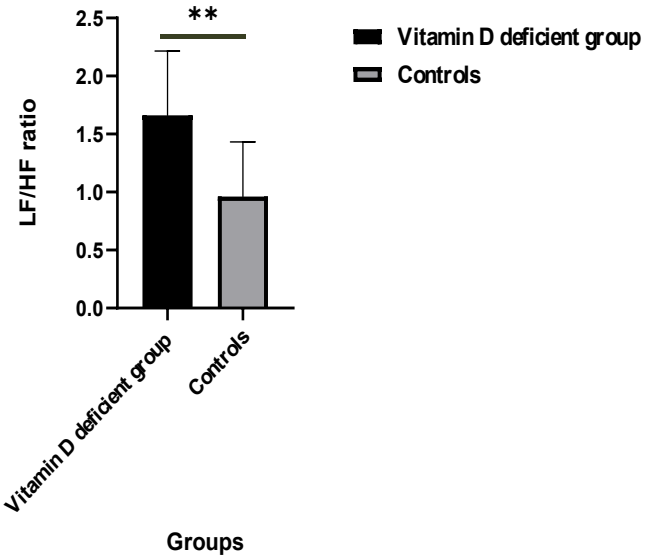

Fig. 2: Differences in LF/HF in individuals with vitamin D deficiency and sufficient vitamin D levels

Evidence from earlier investigations indicates that advancing age is associated with progressive changes in autonomic regulation, characterized by diminished parasympathetic activity together with a relative increase in sympathetic influence [20]. VDRs are circulated within autonomic pathways, and they can traverse the blood–brain barrier and interact with these receptors. Nevertheless, the mechanisms through which VDR activity affects autonomic regulation have not yet been clearly established.

Ageing is characterized by diminished parasympathetic action and relative sympathetic predominance, a pattern that may contribute to alterations in cardiac autonomic function [20]. VDR is identified in the neural and cardiovascular tissues, suggesting a possible contribution to autonomic regulation. However, the exact mechanisms linking vitamin D status with autonomic function remain unclear, and such relationships cannot be established in the present cross-sectional study. Further research incorporating molecular ageing markers, such as telomere length and genetic indicators, may provide deeper insights.

Despite the important findings, certain limitations should be recognized. Cross-sectional design allows identification of associations but does not infer causal relationships between vitamin D status and cardio-metabolic ageing-associated biomarkers. Future longitudinal investigations are required to determine whether vitamin D contributes to the development of alterations in these physiological indicators over time. Information regarding possible confounders, such as habitual physical activity, sun exposure, and dietary vitamin D intake, was not obtained in the present study; therefore, statistical adjustment for these variables could not be performed. These factors may influence both vitamin D concentrations and cardio-metabolic health parameters, and their absence may have contributed to residual confounding. Additionally, the present study measured serum vitamin D using ELISA, whereas reference methods such as LC-MS/MS and validated automated immunoassays provide superior analytical performance. In future investigations, a standardized evaluation of key confounding variables should also be incorporated to enhance the robustness and generalizability of the findings and employ higher-precision analytical methods to strengthen the accuracy and generalizability of results.

Conclusion

This research highlights an association between vitamin D and an adverse cardiometabolic ageing profile, as reflected by alterations in selected biomarkers, when compared with individuals having adequate vitamin D levels. Nevertheless, this study design (cross-sectional) does not permit causal inference. A longitudinal study design is required to determine the directionality of these associations. Further studies including telomere length and relevant genetic indicators, along with longitudinal designs, are warranted to further elucidate the contribution of vitamin D to biological ageing.

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Author’s Contributions

Sumit Kumar: Conceived and designed the study, developed the methodology, conducted the investigation, collected the data, performed the statistical analyses, and prepared the initial version of the manuscript.
Adithi K: Contributed to data validation and organization, performed the literature review, and critically revised the manuscript for important intellectual content.
Shailaja Moodithaya: Provided overall supervision and methodological guidance, critically reviewed the manuscript, and approved the final version for publication.

Conflict of Interest

Authors declared no conflicts of interest.

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