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"A Case-Control Study Identifying Risk Factors Associated with Essential Hypertension"

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Abstract

Hypertension, as a clinical condition, is widely recognized as a significant contributor to premature mortality on a global scale plays an important function in the development of cardiac disorders including heart disease as well as stroke. Vitamin D is a key negative regulator of the Renin-Angiotensin-Aldosterone System (RAAS), a well-known system that regulates blood pressure and determines organ damage. As a result, the main aim of this study was to determine blood calcium and vitamin D levels for individuals with essential hypertension. The research had a total of 328 volunteers, encompassing 164 individuals with hypertension and an equal number of 164 healthy individuals serving as controls. The collection of anthropometric data was carried out following well-established protocols and utilizing precise techniques to ensure accuracy and quality of data obtained. It was found that hypertensive individuals had significantly reduced levels of serum 25-(OH) D and calcium than the control group (18.10±7.88 vs 21.36±8.16; p= 0.000; 8.16±1.20 vs 8.84±1.53; p= 0.000, respectively), underscoring the potential significance of these micronutrients in hypertension. Furthermore, the results of the SEM analysis revealed a significant correlation between calcium and 25-OH vitamin D, indicating both direct and indirect effects on the development of essential hypertension. As a consequence, the study suggests that these biochemical parameters may play a role in the complex control of blood pressure, alongside the results hinting at potential avenues for further exploration through large-scale clinical trials based on the insights from the present study outcomes.

Keywords: 25(OH) vitamin D, Calcium, Essential Hypertension, Renin angiotensin aldosterone system, Structural Equation Modeling.

Introduction

As a clinical entity, Hypertension is widely recognized as a significant contributor to premature mortality and a major contributor to global cardiovascular morbidity [1]. It is the most important public health problem globally, with over 90% of cases due to essential hypertension, a potential threat for cardiovascular disease in the elderly [2, 3]. According to the World Health Statistics 2012, about one billion individuals had hypertension in 2000, expected to rise to 1.56 billion by 2025 [4]. Despite advances in the study regarding its pathophysiology, the etiology remains unclear. Peripheral resistance is one of the determinants of arterial pressure. It is well-known that increasing active tension in the

vascular smooth muscle causes peripheral resistance to rise. Essential hypertension is now considered a complex disease that arises as a result of multiple genetic, environmental, and behavioural factors [5].

Primary hypertension is linked to a halt in the metabolism of calcium. Calcium ions have an important role in the stimulation and contraction of cardiac and smooth muscle cells. Calcium deficiency triggers both PTH and RAAS. Aldosterone enhances the permeability of the apical membrane to Na+, resulting in an augmentation of Na+ and water reabsorption in the kidney's collecting duct cells. This causes an increase in the extracellular fluid volume (ECF), consequently elevating blood pressure and cardiac output [6] (Fig. 1).

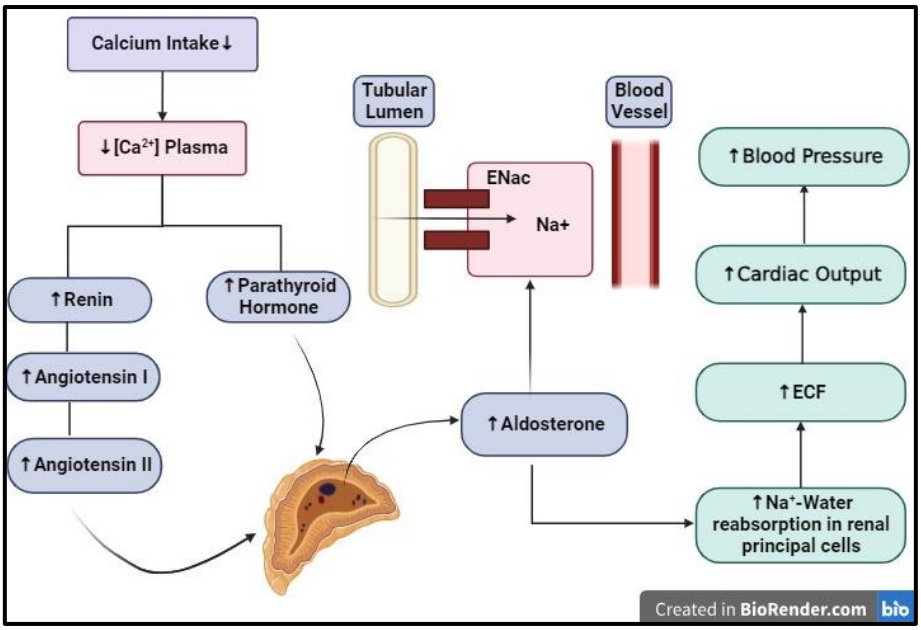

Fig. 1: Proposed mechanism of calcium resulting in increased blood pressure

Vitamin D serves as a fat-soluble vitamin which exists in both forms: ergocalciferol from plants and cholecalciferol from animals [7]. In humans, the skin synthesizes it by converting 7-dehydrocholesterol into pre-vitamin D3 under sun exposure, and then further into vitamin D3 (VD3) [8]. VD3 is carried through the bloodstream by the vitamin D-binding protein (VDBP) and metabolized into 25-OH vitamin D by the enzyme 1, 25 α hydroxylase in liver [9,10]. Subsequently, it undergoes a conversion into 1,25-dihydroxyvitamin D3 by the enzyme 25-hydroxyvitamin D3-1α hydroxylase in kidney [11].

Vitamin D Levels have an inverse relationship to both blood pressure and essential hypertension [12]. Animal and human studies support the possibility that 1,25 (OH)2D3 deficiency makes the RAAS more active both systemically and in the kidney—that is why persons with low levels of 1,25(OH)2D3 may be predisposed to hypertension [13].

Additionally, the VDR is present in the vascular smooth muscle, JG cells of the kidney, and cardio vascular tissues there it directly affects calcium influx, muscle relaxation and diastolic function [14, 15].

The renin-angiotensin-aldosterone is important in the physiology of salt-volume homeostasis. Nonetheless, excessive RAAS also seem to involve other cardiometabolic disorders like hypertension, renal diseases and diabetes etc. On the overall, available evidence indicate that Vitamin D negatively regulates both systemic and local tissue RAAS [16, 17].

Vitamin D contributes to hypertension through various pathways, in addition to the RAAS [6]. Vitamin D is an important regulator of calcium metabolism and disrupted calcium could contribute substantially to the pathophysiology of essential hypertension [18,19]. Vitamin D maintains calcium balance by increasing osteoclastic release of calcium from bones, facilitating renal reabsorption of calcium, and producing more calcium carriers [20, 21].

Given above, the current study was undertaken to assess above mentioned micronutrients and its association among hypertensive cases when compared with normal controls.

Materials And Methods

The study involved 328 participants, including 164 hypertension patients and 164 healthy controls, from the Medicine outpatient department and Medicine wards of the Integral Institute of Medical Sciences and Research in Lucknow. The participants were selected using a non-probability purposive sampling method, taking into account specific inclusion and exclusion criteria and obtained written informed consent.

Criteria For Inclusion and Exclusion Of Study Participants

Patients diagnosed with essential hypertension, specifically within the age range of 35 to 60 years, participated in the study. Patients with a history of diabetes, arthritis, liver disease, kidney disease, cardiovascular disease, and patients taking vitamin D and calcium supplements and antihypertensive drugs were excluded. Pregnant women were also excluded.

Assessments of Covariates

A step-by-step approach based on the NCD Risk Factor Surveillance (STEPS) guidelines was employed to measure weight and height in order to estimate the BMI. Blood pressure was recorded while the participants were in a seated position after resting for 10 minutes, following the criteria outlined in JNC 8. [22]. Demographic details and clinical history were collected through structured Performa for each subject.

Ethics Approval

The Institutional Ethics Committee of IIMS&R has given its approval for the recruitment of human subjects and the collection of blood samples. The number of the institutional ethics committee is IEC/IIMS&R/2022/73.

Sample Collection and Processing

3ml venous blood was collected from each subjects using plain vial and allowed to clot before centrifugation. 0.2 ml serum was used to estimate vitamin D and 0.02 ml serum was used to estimate calcium.

Outcome Definitions

Vitamin D levels less than 10 ng/mL was considered deficient, levels between 10-30 ng/mL as insufficient, 30-100 ng/mL as sufficient, and above 100 ng/mL as indicative of vitamin D toxicity. [Qualisa™ vitamin D ELISA kit [Tulip diagnostic (P) Ltd].

Laboratory Investigations

The assessment of Serum Vitamin D levels was conducted using the Qualisa™ 25-Hydroxyvitamin D ELISA kit, which is commercially provided by Tulip diagnostic (P) Ltd. The measurements were carried out with the thermo scientific multiskan FC ELISA plate reader using the sandwich ELISA method. Calcium levels were determined by o-cresol phthalein Complexone (OCPC) method using semi auto analyzer.

Statistical Analysis

Data analysis was conducted utilizing SPSS-AMOS (26.0) version, which is a widely used software in the field of research. The presentation of all data was in the format of mean ± standard deviation, providing a comprehensive overview of the central tendency and variability within the dataset. Student t-test was employed, allowing for a robust analysis of differences between the two groups. Moreover, a Structural Equation Model (SEM) was meticulously constructed to delve into the Direct, Indirect, and Total Effects of demographic, anthropometric, and biochemical profiles (specifically focusing on vitamin D and calcium) on essential hypertension, shedding light on the intricate relationships among these variables. The criteria for evaluating the SEM model in the study was same as used in previous studies, ensuring the reliability and validity of the model [23, 24].

Our conceptual model provides a detailed representation of the proposed connections between exogenous (independent) and endogenous (dependent) variables, highlighting their intricate association with the outcome of interest, namely essential hypertension as shown in Figure 2. Within this conceptual framework, the exogenous variables encompass

Demographic Profile factors like Gender and Age, Anthropometric Profile measures including BMI and WHR, and Biochemical Profile components such as Vitamin D and calcium. Conversely, the endogenous variables pertain to SBP & DBP, crucial factors in understanding hypertension. Subsequently, translation of the conceptual model into a statistical model was meticulously carried out, involving the application of confirmatory factor analysis (CFA) to establish constructs or factors and the development of an initial SEM. The main objective of the confirmatory factor analysis was to assess the degree to which the data correspond to the proposed measurement model, ensuring the reliability of the subsequent analyses.

Moving forward, in the subsequent stage, we thoroughly examine the conceptual model and reveal the intricate relationships between variables linked to essential hypertension. Within the SEM framework, the unidirectional arrows represent causal relationships or regressions, while the bidirectional arrows indicate correlations between variables, allowing for a comprehensive exploration of these connections. The Structural Equation Modeling (SEM) enabled a comprehensive investigation into the direct and indirect connections among the variables associated with essential hypertension. This examination provided valuable insights into the underlying mechanisms.

Additionally, parameters like the Root Mean Square Residual (RMR) were employed to assess the disparities between the observed and model-predicted covariance matrices. The Chi-Square to Degrees of Freedom Ratio (χ^2/df) was used to estimate chi-square values, while the Root Mean Square Error of Approximation (RMSEA) measured the differences between the model and observed covariance matrices, considering the complexity of the model. Lastly, P Close value, representing the p-value associated with the chi-square statistic, played a crucial role in determining the statistical significance of the model fit, with p-values below 0.05 indicating a noteworthy fit.

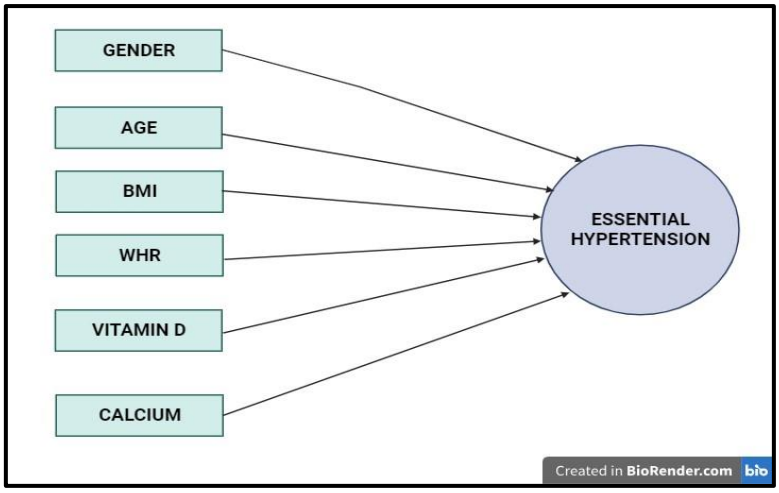

Figure 2: Conceptual model showing the association of Demographic, Anthropometric, and Biochemical Profiles with Hypertension

Results

A total of 328 participants were included in this case-control research. The findings of the data analysis have been outlined in the tables. Table 1 presents the descriptive analysis of the study variables between both groups. The statistical analysis revealed that calcium and vitamin D levels were notably decreased in cases compared to the controls (18.10±7.88 vs 21.36±8.16; p= <0.001; 8.16±1.20 vs 8.84±1.53; p= <0.001).

Table 1: Characteristics of the study Subjects

Demographic, Anthropometric, & Biochemical Profile	Cases (Mean± S.D) N=164	Controls (Mean± S.D) N=164	p-value (<0.05)
Age (years)	45.26±5.47	43.78±6.96	0.033*
Gender, n (%)			
Male	81(49.39%)	83(50.61%)	1.000
Female	81(49.39%)	83(50.61%)	1.000
BMI(Kg/m2)	24.38±3.56	23.22±3.47	0.003*
WHR (W/H)	0.90±0.03	0.91±0.03	0.003*
SBP (mmHg)	154.54±12.53	120.70±9.41	<0.001
DBP (mmHg)	93.39±6.37	76.15±7.18	<0.001
25-(OH)D (ng/ml)	18.10±7.88	21.36±8.16	<0.001
Calcium (mg/dl)	8.16±1.20	8.84±1.53	<0.001
*p<0.05 is statistically significant			

SEM model indicated a significant inverse association of vitamin D and calcium with essential hypertension. All Indices in the model suggest final model fits the data well, displayed in Table 2 and Figure 3. Additionally, a positive correlation between calcium and vitamin D was seen among those with high blood pressure (see Table 3) The model's

estimates reveal the direct, indirect, and overall impact of independent variables on positive outcomes such as essential hypertension (see Table 4 and Figure 4)

Table 2: Estimates of CFA Model Fit

Measure	Estimate	Threshold
χ^2/df	.864	<3
GFI	.991	>0.90
AGFI	.953	>0.90
NFI	.953	>0.90
SRMR	1.308	<0.08
RMSEA	.000	≤0.05

χ^2/df: Chi-Square to Degrees of Freedom Ratio; GFI: Goodness of Fit Index; AGFI: Adjusted Goodness of fit Index; NFI: Normed Fit Index; SRMR: Standardized Root Mean Square Residual; RMSEA: Root Mean Square Error of Approximation.

Table 3: Estimates of correlation of demographic, anthropometric, and biochemical parameters in essential hypertension cases.

Variables			Estimate
Gender	<---------->	BMI	.193
Age	<---------->	Gender	-.014
Age	<---------->	BMI	.210
WHR	<---------->	Age	.062
WHR	<---------->	Gender	.037
WHR	<---------->	BMI	.122
Calcium	<---------->	Gender	.036
Calcium	<---------->	WHR	-.130
Calcium	<---------->	BMI	-.084
Calcium	<---------->	Age	-.255
Vitamin D	<---------->	Gender	-.015
Vitamin D	<---------->	Age	-.518
Vitamin D	<---------->	BMI	-.056
Vitamin D	<---------->	WHR	-.187
Vitamin D	<---------->	Calcium	.174
Essential Hypertension	<---------->	Calcium	-.209
Essential Hypertension	<---------->	Vitamin D	-.330
Essential Hypertension	<---------->	Gender	.248

Essential Hypertension	<----------> Age	.176
Essential Hypertension	<----------> BMI	.210
Essential Hypertension	<----------> WHR	.284

Table 4: Effects of Independent Variables on Essential Hypertension.

Variables	Direct Effect	Indirect Effect	Total Effect
Gender	-.040	.220	.180
Age (years)	.217	.033	.251
BMI(Kg/m2)	.126	.010	.135
WHR (W/H)	.192	.062	.254
25-(OH)D (ng/ml)	-.280	-.005	-.285
Calcium	-.143	.000	-.143

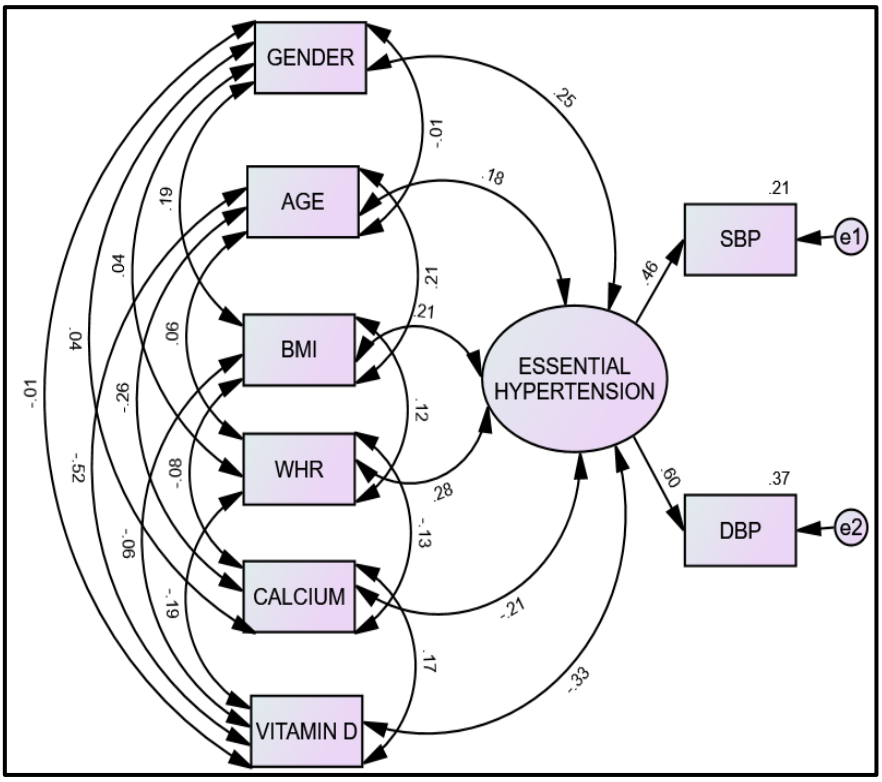

Figure.3: Structural Equation Model (SEM) with an estimate of CFA of Demographic, anthropometric, and Biochemical parameters with essential hypertension

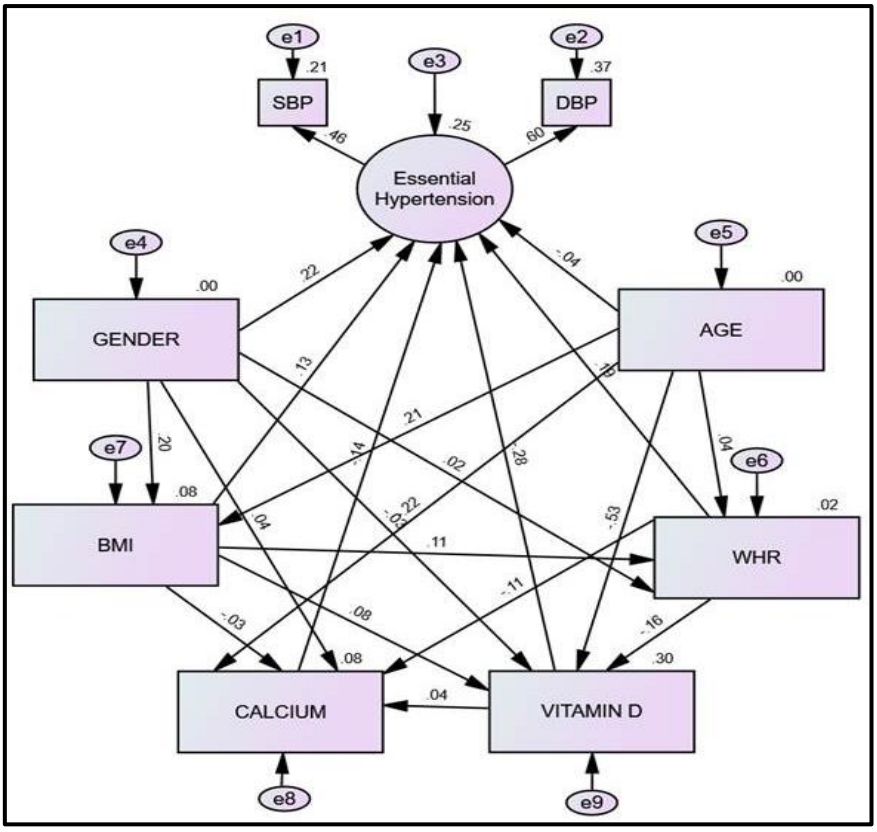

Figure 4: Structural Equation Model (SEM) with estimates of Direct, Indirect, and Total Effect of Independent variables on Essential Hypertension

Discussion

This study explored intricate relationships between variables linked to essential hypertension with a significant change in micronutrient levels. SEM model used in the study demonstrates an exceptionally good fit for the data. The extremely low RMR, CMIM/DF, and RMSEA values, along with the perfect CFI and high P Close, all indicate a very strong fit. These results suggest that the model accurately explains the relationships among the variables. The model's estimates reveal the direct, indirect, and overall impact of independent variables on positive outcomes such as essential hypertension. Our findings are in continuation with results of previous studies conducted by [4, 25]. Hypertension is a multifactorial condition in which different physiological mechanisms work together to raise blood pressure. Many hypotheses concerning the probable mechanism underlying critical hypertension have been postulated, including electrolyte imbalance. Recent research found that calcium plays an important role in essential hypertension pathogenesis however, findings were inconclusive [26].

Several epidemiological studies have associated low serum total and ionized calcium levels, elevated intracellular calcium, increased urinary calcium excretion, and higher concentrations

of parathyroid hormone (PTH) with essential hypertension in both humans and genetic animal models of hypertension [27]. McCarron *et al.,* observed lower total Ca levels in patients with high blood pressure compared to healthy individuals [28]. Conversely, Hazari *et al*., discovered no significant disparity in total serum Ca levels between both groups [19].

Numerous studies have identified a link between total serum calcium and blood pressure. However, not all investigations agreed; a few studies revealed lower levels of ionized calcium in hypertensive individuals even if their total calcium levels are comparable. According to some authors, calcium intake in the diet is independent of blood pressure levels. However, reduced calcium levels in the diet may involve in the pathogenesis and development of hypertension. These views were supported by research that demonstrated decreased levels of blood pressure in people who were given calcium supplements after being diagnosed with hypertension [29].

Another factor that has received attention in regards to the onset of hypertension is vitamin D. For decades, vitamin D metabolites have been linked to blood pressure control and hormonal systems that regulate blood pressure [30]. The primary connection between vitamin D and hypertension is as a negative regulator of the RAAS, which is a well-known mechanism. According to other established hypotheses, vitamin D may influence vascular endothelial function (31). Due to the fact that increased RAAS activity is linked to increase in blood pressure and cardiovascular risk [32], many studies have been conducted to explore the exact mechanism linking vitamin D and in hypertension.

Studies linking vitamin D to RAAS activity and blood pressure regulation have been conducted in animal models. Li *et al.,* found that VDR knockout mice showed increased renin activity and angiotensin II levels, leading to high blood pressure and heart enlargement [33]. These issues could be reduced with RAAS antagonist drugs and increased cardiac tissue RAAS activity [33,34].

Furthermore, a mouse model lacking 1-hydroxylase activity, which results in the inability to produce 1,25(OH)2D, displayed symptoms such as increased RAAS function, high blood pressure, and ventricular enlargement. These symptoms were improved by administering either 1,25(OH)2D or RAAS inhibitors. The research conducted by Zhou *et al.,* also demonstrated that the activation of VDR by 1,25(OH)2D limits the renin gene expression, suggesting that VDR agonists could potentially offer protection against high blood pressure and cardiac issues [35].

The initial randomized trial showed that a single dose of 100,000 IU of vitamin D3 did not reduce blood pressure compared to a placebo after 5 weeks [36]. However, the brief duration

and slight rise in 25(OH)D levels cast doubt on the negative outcome. A subsequent study found that older women deficient in 25(OH)D experienced a modest drop in blood pressure, but the short follow-up period and small sample size weakened the significance of these findings [37].

Large-scale randomized trials are needed to assess the impact of vitamin D treatment on blood pressure, as most clinical data comes from cross-sectional or interventional studies with design flaws. Future interventional studies should have a large sample size, extended follow-up period, higher vitamin D supplementation doses, and limitations on antihypertensive medications, while accounting for RAS confounders.

Conclusion

The study shows that individuals with essential hypertension have significantly reduced vitamin D and calcium compared to healthy controls. The relationship between these micronutrients and hypertension is complex, with vitamin D influencing essential hypertension through both direct and indirect pathways, and calcium having a direct impact only. Maintaining adequate levels of these nutrients may be crucial for prevention and management of essential hypertension. Future research should explore the underlying mechanisms and therapeutic implications.

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Author Contributions

The study was conceptualized and planned by RA, PTM, RKK, DT, and R ALAM, followed by data acquisition by RA and RKK, with tables and figures designed by PTM, RA, and DT, and all authors involved in the interpretation and revision.

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Nil

Conflict of Interest

Authors declares no conflict of Interest Exist.

Data Availability

All data used in the study are included in this manuscript. All the materials and reagent sources used in this study are described in the materials & methods section.

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