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Original Research Article

A Multidisciplinary Evaluation of Endothelial Dysfunction, Gut Dysbiosis, Inflammatory Biomarkers, and Their Association with Essential Hypertension: A Case–Control Study

Nazir Ahmad Var[1], Kashif Naem Siddiqqi[2], Nishesh Singh Kotwal[3]

[1]Associate Professor, Department of Microbiology, Medical College, (KD University), Mathura UP India
[2]Assistant Professor, Department of Biochemistry, MMC, Madhubani, Bihar India
[3]Senior Resident, Department of Physiology, Govt. Medical College Doda J&K, India

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Corresponding author: Dr. Nishesh Singh Kotwal
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Abstract

Background: Essential hypertension remains one of the most prevalent non-communicable diseases worldwide and is a major contributor to cardiovascular morbidity and premature mortality. While sustained elevation of arterial blood pressure has traditionally been attributed to neurohormonal activation, renal sodium handling, and vascular remodeling, accumulating evidence suggests that endothelial dysfunction, chronic low-grade systemic inflammation, and alterations in gut microbial ecology collectively contribute to disease initiation and progression. The interaction between vascular endothelial integrity, immune activation, and the gut microbiome has emerged as an important mechanistic pathway linking environmental and metabolic risk factors with persistent hypertension. However, multidisciplinary evaluation integrating these biological domains within a single clinical population remains limited, particularly in hospital-based settings.

Objectives: The present analytical case–control study was undertaken to comprehensively evaluate endothelial dysfunction, gut dysbiosis, and inflammatory biomarkers among patients with essential hypertension and to determine their independent and combined associations with disease severity.

Methods: A hospital-based analytical case–control study was conducted over an 18-month period in a tertiary care teaching hospital. A total of 320 participants were enrolled, comprising 160 clinically diagnosed patients with essential hypertension (cases) and 160 age- and sex-matched normotensive healthy individuals (controls). All participants underwent detailed clinical examination, anthropometric assessment, blood pressure profiling, biochemical investigations, endothelial function analysis, inflammatory biomarker estimation, and gut microbiome evaluation. Endothelial function was assessed by serum nitric oxide (NO), endothelin-1 (ET-1), vascular cell adhesion molecule-1 (VCAM-1), intracellular adhesion molecule-1 (ICAM-1), and brachial artery flow-mediated dilatation (FMD). Inflammatory status was evaluated using high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and monocyte chemoattractant protein-1 (MCP-1). Gut microbial alterations were assessed through quantitative polymerase chain reaction (qPCR)-based estimation of dominant bacterial taxa, Firmicutes/Bacteroidetes ratio, relative abundance of Lactobacillus spp. and Bifidobacterium spp., serum lipopolysaccharide (LPS), and fecal calprotectin. Multivariate logistic regression, Pearson's correlation analysis, receiver operating characteristic (ROC) curve analysis, and multiple linear regression models were employed for statistical evaluation.

Results: Compared with healthy controls, hypertensive patients demonstrated significantly reduced endothelial function, evidenced by lower serum nitric oxide levels and impaired flow-mediated dilatation, accompanied by significantly elevated endothelin-1, VCAM-1, and ICAM-1 concentrations ($p < 0.001$). Inflammatory biomarkers including hs-CRP, IL-6, TNF-α, and MCP-1 were markedly increased among hypertensive individuals, indicating persistent systemic inflammatory activation. Gut microbiome analysis revealed significant dysbiosis characterized by:

- Reduced abundance of beneficial Lactobacillus and Bifidobacterium species
- Increased Firmicutes/Bacteroidetes ratio
- Elevated serum lipopolysaccharide concentrations
- Higher fecal calprotectin levels

Serum lipopolysaccharide demonstrated strong positive correlations with hs-CRP, IL-6, endothelin-1, and systolic blood pressure while showing an inverse relationship with nitric oxide bioavailability and flow-mediated dilatation.

Multivariate regression identified endothelial dysfunction markers, serum lipopolysaccharide, IL-6, and reduced nitric oxide levels as independent predictors of essential hypertension after adjustment for age, sex, body mass index, diabetes, smoking, and dyslipidemia. Receiver operating characteristic analysis demonstrated that

combined assessment of nitric oxide, endothelin-1, IL-6, and serum lipopolysaccharide significantly improved discrimination between hypertensive and normotensive individuals compared with individual biomarkers alone.
Conclusion: The study showed that endothelial dysfunction, gut microbial dysbiosis, and chronic systemic inflammation coexist in patients with essential hypertension and exhibit strong interdependent associations. These findings support the concept of a gut–vascular–immune axis contributing to hypertension pathogenesis and suggest that integrated biomarker profiling may facilitate improved risk stratification and early identification of vascular dysfunction. Therapeutic strategies targeting restoration of endothelial homeostasis and modulation of gut microbial composition may represent promising adjunctive approaches for the prevention and management of essential hypertension.
Keywords: Essential Hypertension; Endothelial Dysfunction; Gut Microbiota; Gut Dysbiosis; Nitric Oxide; Endothelin-1; Inflammatory Biomarkers; Interleukin-6; Tumor Necrosis Factor-Alpha; Lipopolysaccharide; Vascular Inflammation; Flow-Mediated Dilatation; Microbiome; Chronic Inflammation; Cardiovascular Risk.
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Introduction

Essential hypertension is among the most important modifiable determinants of cardiovascular disease and remains a leading cause of global morbidity, disability, and premature mortality. It affects more than one billion adults worldwide and accounts for a substantial proportion of ischemic heart disease, stroke, chronic kidney disease, heart failure, peripheral arterial disease, and vascular dementia [1]. Despite considerable advances in antihypertensive pharmacotherapy, blood pressure control remains suboptimal in many populations, indicating that the underlying biological mechanisms extend beyond conventional hemodynamic abnormalities [2].

Traditionally, the pathogenesis of essential hypertension has been attributed to excessive activation of the renin–angiotensin–aldosterone system, increased sympathetic nervous system activity, altered renal sodium handling, endothelial dysfunction, and structural vascular remodeling [3]. These mechanisms collectively contribute to sustained elevation of systemic vascular resistance and impaired cardiovascular homeostasis [4]. However, contemporary research indicates that hypertension is increasingly recognized as a multifactorial inflammatory vascular disorder involving complex interactions among metabolic, immunological, microbial, and endothelial pathways [4]. Among these mechanisms, endothelial dysfunction occupies a central position in the development of hypertension [5]. The vascular endothelium is an active endocrine and paracrine organ that regulates vascular tone, platelet activity, coagulation, inflammation, oxidative stress, angiogenesis, and smooth muscle proliferation. Physiologically, endothelial cells maintain vascular homeostasis through continuous production of vasodilatory mediators, including nitric oxide (NO), prostacyclin, and endothelium-derived hyperpolarizing factor, while simultaneously suppressing inflammatory activation and thrombogenesis [6]. Under pathological conditions, excessive oxidative stress and inflammatory activation reduce nitric oxide bioavailability through increased generation of reactive oxygen species [7]. This imbalance favors production of potent vasoconstrictors such as endothelin-1 [8], resulting in impaired vasodilatation, enhanced vascular stiffness, leukocyte adhesion, platelet activation, and progressive arterial remodeling [9].

Adhesion molecules including vascular cell adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1) facilitate endothelial inflammation and leukocyte recruitment, thereby accelerating vascular injury [10]. Increasing attention has also been directed toward the role of chronic low-grade systemic inflammation in hypertension [11]. Persistent activation of innate and adaptive immune responses contributes to endothelial injury through continuous production of pro-inflammatory cytokines, including interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), monocyte chemoattractant protein-1 (MCP-1), and high-sensitivity C-reactive protein (hs-CRP). These mediators promote oxidative stress, vascular smooth muscle proliferation, extracellular matrix deposition, and arterial stiffness, thereby perpetuating elevated blood pressure [12]. Inflammatory activation is now recognized not merely as a consequence of hypertension but as an active participant in disease initiation and progression [13].

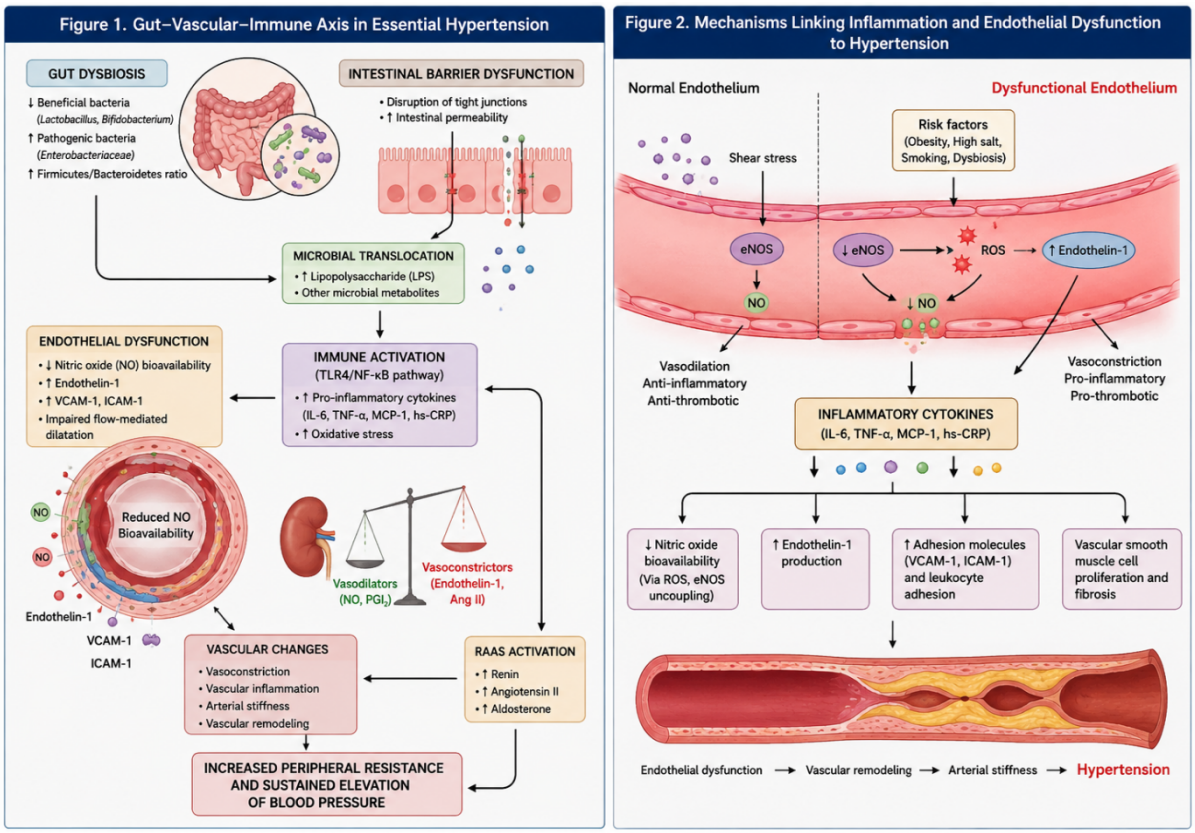

Figure 1:

An equally important emerging concept is the influence of the gut microbiome on cardiovascular regulation. The human gastrointestinal tract contains trillions of microorganisms that collectively participate in nutrient metabolism, immune modulation, maintenance of epithelial barrier integrity, and production of bioactive metabolites. A balanced microbial ecosystem contributes to metabolic homeostasis through generation of short-chain fatty acids, regulation of bile acid metabolism, and modulation of host inflammatory responses.

Disruption of this microbial balance, commonly referred to as gut dysbiosis, has been implicated in several cardiometabolic disorders, including obesity, diabetes mellitus, metabolic syndrome, atherosclerosis, chronic kidney disease, and essential hypertension. Hypertensive individuals frequently exhibit reduced microbial diversity, depletion of beneficial genera such as Lactobacillus and Bifidobacterium, expansion of opportunistic bacterial populations, and an increased Firmicutes/Bacteroidetes ratio [14].

These alterations may compromise intestinal epithelial barrier function, facilitating translocation of bacterial lipopolysaccharide into the systemic circulation [15]. Circulating lipopolysaccharide activates Toll-like receptor-4 signaling pathways, amplifying systemic inflammation, oxidative stress, endothelial dysfunction, and vascular remodeling [16]. The concept of a gut–vascular–immune axis provides a unifying framework for understanding how microbial alterations influence blood pressure regulation [17]. Microbial metabolites, inflammatory cytokines, endothelial mediators, and neurohormonal pathways interact dynamically, creating a self-perpetuating cycle of vascular dysfunction [18]. Reduced production of protective short-chain fatty acids diminishes anti-inflammatory signaling and endothelial nitric oxide synthesis, whereas increased endotoxemia enhances cytokine release, reactive oxygen species generation, and vasoconstrictor production [19].

Although several investigations have independently examined endothelial biomarkers, inflammatory mediators, or gut microbiota in hypertension, few studies have simultaneously evaluated these interconnected pathways within the same patient population using a multidisciplinary analytical approach. Consequently, the relative contributions and interactions among endothelial injury, microbial dysbiosis, and systemic inflammation remain incompletely characterized in routine clinical settings [20].

The present hospital-based analytical case–control study was therefore designed to comprehensively investigate endothelial function, inflammatory biomarkers, and gut microbial alterations among patients with essential hypertension. By integrating clinical assessment with vascular physiology,

immunology, microbiology, and biochemical analyses, this study aims to provide a holistic understanding of the biological mechanisms underlying essential hypertension [21]. Furthermore, the study seeks to identify novel biomarker combinations capable of improving early detection, risk stratification, and future therapeutic targeting of hypertensive vascular disease.

Aim of the Study: To comprehensively evaluate endothelial dysfunction, gut microbial dysbiosis, and inflammatory biomarkers among patients with essential hypertension and to determine their independent and combined associations with the presence and severity of disease in a hospital-based analytical case–control study.

Primary Objectives

1. To compare endothelial function biomarkers between hypertensive patients and normotensive controls.
2. To evaluate circulating inflammatory biomarkers in essential hypertension.
3. To assess gut microbial alterations and markers of intestinal permeability.
4. To determine the association between gut dysbiosis and endothelial dysfunction.
5. To evaluate correlations between inflammatory biomarkers and blood pressure indices.
6. To identify independent predictors of essential hypertension using multivariable statistical models.

Secondary Objectives

- To assess the relationship between serum lipopolysaccharide and endothelial dysfunction.
- To determine whether combined biomarker profiling improves discrimination of hypertensive individuals.
- To explore interactions among microbial, inflammatory, and endothelial parameters within the gut–vascular–immune axis.

Research Hypothesis

Null Hypothesis (H_0): There is no significant association between endothelial dysfunction, gut dysbiosis, inflammatory biomarkers, and essential hypertension.

Alternative Hypothesis (H_1): Patients with essential hypertension exhibit significant endothelial dysfunction, gut microbial dysbiosis, and chronic systemic inflammation, and these factors independently and synergistically contribute to the pathogenesis of essential hypertension.

Research Methodology

Study Design: The case–control design was selected because it permits simultaneous evaluation of multiple biological variables associated with hypertension and facilitates comparison between hypertensive patients and normotensive healthy controls under similar environmental and demographic conditions.

The investigation was carried out over a period of 18 months, including participant recruitment, clinical evaluation, laboratory investigations, microbiological analysis, data validation, statistical analysis, and interpretation of findings.

Study Setting: The study was conducted in a tertiary care teaching hospital attached to a medical college. All laboratory investigations were performed using standardized operating procedures to minimize analytical variability.

Study Population: The study population consisted of adult individuals aged between 30 and 65 years.

Two groups were enrolled:

Case Group: Patients diagnosed with essential hypertension according to current international diagnostic criteria and receiving evaluation in the Department of General Medicine.

Control Group: Age- and sex-matched apparently healthy normotensive volunteers without previous history of hypertension or cardiovascular disease.

Sample Size Determination: The sample size was calculated assuming a moderate effect size for endothelial dysfunction markers between hypertensive and normotensive populations.

Assumptions included:

- Confidence level = 95%
- Statistical power = 80%
- Expected effect size = 0.5
- Case-control ratio = 1:1

The minimum required sample size was estimated to be 146 participants per group. To compensate for potential exclusions, incomplete laboratory investigations, and specimen inadequacy, the sample size was increased by approximately 10%.

Accordingly,

Cases = 160

Controls = 160

Total sample size = 320 participants

This sample size provided adequate statistical power for multivariate regression analyses and subgroup comparisons.

Sampling Technique: A consecutive purposive sampling technique was adopted. Eligible hypertensive patients attending the Medicine outpatient department or admitted to inpatient wards during the study period were screened for eligibility. Participants fulfilling the inclusion

criteria and providing written informed consent were enrolled until the desired sample size was achieved.

Healthy controls were recruited from hospital staff, patient attendants, and community volunteers after confirmation of normotensive status.

Inclusion Criteria

Cases: Participants fulfilling all the following criteria were included:

- Age between 30 and 65 years.
- Diagnosed with essential hypertension according to current clinical guidelines.
- Either newly diagnosed or receiving stable antihypertensive therapy for at least three months.
- Ability to provide informed written consent.
- Willingness to provide blood and stool samples.

Controls:

Healthy volunteers satisfying the following criteria were enrolled:

- Age between 30 and 65 years.
- Systolic blood pressure below 120 mmHg.
- Diastolic blood pressure below 80 mmHg.
- No previous diagnosis of hypertension.
- No history of cardiovascular disease.
- Apparently healthy on clinical examination.
- Written informed consent.

Exclusion Criteria: Participants were excluded if any of the following conditions were present:

- Secondary hypertension.
- Diabetes mellitus requiring insulin therapy.
- Chronic kidney disease (Stage III or higher).
- Liver cirrhosis.
- Heart failure.
- Acute coronary syndrome.
- Stroke.
- Active infection within the preceding four weeks.
- Autoimmune disease.
- Malignancy.
- Pregnancy.
- Inflammatory bowel disease.
- Recent gastrointestinal surgery.
- Antibiotic or probiotic use during the previous eight weeks.
- Chronic corticosteroid therapy.
- Immunosuppressive medications.
- Chronic alcohol dependence.
- Refusal to participate.

Participant Recruitment: A total of 402 individuals were initially screened.

Following eligibility assessment:

- 178 hypertensive patients fulfilled inclusion criteria.
- 170 healthy volunteers fulfilled inclusion criteria.

After applying exclusion criteria and accounting for incomplete investigations:

- 160 hypertensive patients were included.
- 160 healthy controls were included.

Thus,

Final sample = 320 participants.

Clinical Evaluation: Each participant underwent standardized clinical examination conducted by trained physicians.

Anthropometric Assessment: Measurements were obtained according to international guidelines.

Height: Measured using a wall-mounted stadiometer. Recorded to the nearest 0.1 cm.

Weight: Measured using calibrated digital weighing scales. Recorded to the nearest 0.1 kg.

Body Mass Index: Calculated as

$$BMI = \frac{\text{Weight (kg)}}{\text{Height (m}^2\text{)}}$$

Participants were classified according to WHO recommendations.

Waist Circumference: Measured midway between the lower rib and iliac crest using a non-stretchable measuring tape.

Hip Circumference: Measured at the widest part of the buttocks.

Waist-Hip Ratio: Calculated using standard formula.

Blood Pressure Measurement: Blood pressure was measured following European Society of Hypertension recommendations.

Participants:

- Avoided caffeine for 30 minutes.
- Avoided smoking for 30 minutes.
- Rested quietly for 10 minutes.
- Remained seated with back supported.

Three readings were recorded using a validated automated sphygmomanometer. Average of the final two readings was considered for statistical analysis.

Measured variables included:

- Systolic Blood Pressure
- Diastolic Blood Pressure
- Mean Arterial Pressure
- Pulse Pressure

Laboratory Investigations: Following overnight fasting (10–12 hours), 15 mL of venous blood was collected under aseptic precautions. Samples were processed within one hour. Serum and plasma aliquots were stored at −80°C until analysis.

Routine Biochemical Investigations

The following parameters were estimated:

- Complete blood count
- Fasting plasma glucose
- HbA1c
- Blood urea
- Serum creatinine
- Estimated GFR
- Serum electrolytes
- Total cholesterol
- HDL cholesterol
- LDL cholesterol
- Triglycerides
- Liver function tests
- Serum uric acid

All analyses were performed using an automated clinical chemistry analyzer with internal and external quality control.

Assessment of Endothelial Dysfunction: The following endothelial biomarkers were evaluated: Serum Nitric Oxide. Estimated indirectly by measuring nitrate/nitrite concentrations using the Griess reaction [22].

Results expressed as:

- µmol/L
- Endothelin-1

Measured using a high-sensitivity sandwich ELISA. Results expressed in: pg/mL

VCAM-1: Measured using commercially validated ELISA kits. Results: ng/mL.

ICAM-1: Estimated using enzyme-linked immunosorbent assay.

Flow-Mediated Dilatation (FMD): Endothelial-dependent vasodilatation was assessed by high-resolution ultrasound of the brachial artery.

The procedure included:

- Baseline arterial diameter measurement.
- Forearm cuff inflation to 50 mmHg above systolic pressure for 5 minutes.
- Reactive hyperemia following cuff release.
- Maximum arterial diameter measured 60–90 seconds after cuff deflation.

Measurement of Inflammatory Biomarkers: The following inflammatory biomarkers were quantified: High-Sensitivity CRP Measured by immunoturbidimetric assay.

IL-6: Measured using quantitative ELISA.

TNF-α: Measured by sandwich ELISA.

MCP-1: Measured using commercially validated ELISA kits. All assays were performed in duplicate.

Gut Microbiome Evaluation: Fresh stool specimens were collected in sterile containers and transported to the microbiology laboratory within two hours. Samples were immediately stored at −80°C.

DNA Extraction: Microbial DNA was extracted using a commercially validated stool DNA extraction kit following the manufacturer's instructions. DNA purity and concentration were assessed using spectrophotometry.

Quantitative PCR Analysis: Species-specific primers were used for quantification of:

- Lactobacillus spp.
- Bifidobacterium spp.
- Escherichia coli
- Firmicutes
- Bacteroidetes

Relative abundance was calculated using the comparative Ct method.

Firmicutes/Bacteroidetes Ratio: The ratio was calculated for each participant as an indicator of microbial dysbiosis.

Intestinal Permeability Markers

Serum Lipopolysaccharide (LPS): Measured using chromogenic Limulus Amebocyte Lysate assay.

Fecal Calprotectin: Measured using quantitative ELISA.

Quality Assurance: To ensure validity and reproducibility:

- All instruments were calibrated daily.
- Laboratory personnel were blinded to participant groups.
- Duplicate assays were performed for biomarker estimation.
- Internal quality controls were included in every analytical run.
- External proficiency testing programs were followed.
- Ten percent of samples were randomly reanalyzed for reproducibility.

Statistical Analysis: Statistical analysis was performed using IBM SPSS Statistics (Version 29.0) and R Statistical Software (Version 4.3). Continuous variables were expressed as mean ± standard deviation (SD) or median (interquartile range), depending on data distribution. Categorical variables were summarized as frequencies and percentages.

Data normality was assessed using the Shapiro–Wilk test. Group comparisons employed the independent t-test for normally distributed variables and the Mann–Whitney U test for non-normal distributions. Associations between categorical variables were evaluated using the Chi-square or Fisher's exact test. Pearson's or Spearman's correlation coefficients were calculated to examine relationships among endothelial biomarkers, inflammatory markers, gut microbiota indices, and blood pressure parameters. To identify independent determinants of essential hypertension, multivariable binary logistic regression was performed after adjustment for potential confounders including age, sex, body mass index, smoking status, dyslipidemia, and physical activity. Results were reported as adjusted odds ratios (AORs) with 95% confidence intervals.

Multiple linear regression models were used to evaluate predictors of systolic blood pressure and endothelial function. Receiver operating characteristic (ROC) curve analysis assessed the diagnostic performance of individual biomarkers and combined biomarker panels, with calculation of the area under the curve (AUC), sensitivity, specificity, and optimal cut-off values.

Model calibration was examined using the Hosmer–Lemeshow goodness-of-fit test, and multicollinearity was assessed through variance inflation factors.

A two-tailed p-value < 0.05 was considered statistically significant throughout the analysis.

Observations and Results

Participant Flow: A total of 402 individuals were screened for eligibility during the study period. After applying the inclusion and exclusion criteria, 348 participants met the eligibility requirements. Twenty-eight eligible individuals declined participation or did not complete the required investigations. Consequently, 320 participants completed all study procedures and were included in the final analysis, comprising 160 patients with essential hypertension (cases) and 160 age- and sex-matched normotensive healthy controls.

No participant was excluded after completion of laboratory investigations because all collected samples satisfied predefined quality-control standards.

Baseline Characteristics of Study Participants

Table 1: Demographic and Clinical Characteristics of Cases and Controls

Variable	Cases (n = 160)	Controls (n = 160)	p-value
Age (years)	52.6 ± 8.4	51.8 ± 7.9	0.412
Male, n (%)	94 (58.8)	91 (56.9)	0.731
Female, n (%)	66 (41.2)	69 (43.1)	0.731
BMI (kg/m²)	28.9 ± 3.5	24.8 ± 2.9	<0.001
Waist Circumference (cm)	97.4 ± 9.1	87.6 ± 7.8	<0.001
Waist–Hip Ratio	0.96 ± 0.05	0.89 ± 0.04	<0.001
Current Smokers, n (%)	49 (30.6)	28 (17.5)	0.009
Alcohol Consumption, n (%)	43 (26.9)	26 (16.3)	0.021
Sedentary Lifestyle, n (%)	101 (63.1)	54 (33.8)	<0.001
Positive Family History of Hypertension, n (%)	92 (57.5)	46 (28.8)	<0.001

Observation:

The two study groups were comparable with respect to age and sex distribution, indicating successful matching. However, participants with essential hypertension showed significantly higher body mass index, greater central adiposity, higher prevalence of smoking, alcohol use, sedentary behavior, and a stronger family history of hypertension than healthy controls.

Blood Pressure Characteristics

Table 2: Blood Pressure Profile of Study Participants

Parameter	Cases	Controls	p-value
Systolic Blood Pressure (mmHg)	151.8 ± 11.9	116.2 ± 7.4	<0.001
Diastolic Blood Pressure (mmHg)	94.7 ± 7.6	73.4 ± 5.3	<0.001
Mean Arterial Pressure (mmHg)	113.7 ± 8.2	87.7 ± 5.4	<0.001
Pulse Pressure (mmHg)	57.1 ± 8.4	42.8 ± 6.1	<0.001
Heart Rate (beats/min)	82.6 ± 9.1	74.2 ± 7.6	<0.001

Observation: As expected, all blood pressure indices were significantly elevated among hypertensive participants. Pulse pressure and mean arterial pressure were also substantially higher, indicating increased arterial stiffness and vascular resistance.

Routine Biochemical Profile

Table 3: Routine Laboratory Investigations

Parameter	Cases	Controls	p-value
Fasting Plasma Glucose (mg/dL)	102.4 ± 11.3	94.8 ± 9.5	<0.001
HbA1c (%)	5.8 ± 0.4	5.4 ± 0.3	<0.001
Total Cholesterol (mg/dL)	213.5 ± 35.7	181.6 ± 28.3	<0.001
LDL Cholesterol (mg/dL)	136.4 ± 28.1	108.3 ± 23.6	<0.001
HDL Cholesterol (mg/dL)	41.2 ± 7.4	51.8 ± 8.2	<0.001
Triglycerides (mg/dL)	176.5 ± 48.2	128.4 ± 34.6	<0.001
Serum Creatinine (mg/dL)	0.98 ± 0.19	0.90 ± 0.17	0.003
eGFR (mL/min/1.73 m²)	92.6 ± 12.4	98.9 ± 11.3	<0.001

Observation

Hypertensive patients exhibited a less favorable metabolic profile, characterized by higher fasting glucose, HbA1c, total cholesterol, LDL cholesterol, triglycerides, and lower HDL cholesterol. Renal function remained within normal limits in both groups, although a modest reduction in estimated glomerular filtration rate was observed among cases.

Endothelial Function Biomarkers

Table 4: Endothelial Biomarkers in Cases and Controls

Biomarker	Cases	Controls	p-value
Nitric Oxide (µmol/L)	22.8 ± 6.3	36.9 ± 7.1	<0.001
Endothelin-1 (pg/mL)	5.82 ± 1.21	2.41 ± 0.76	<0.001
VCAM-1 (ng/mL)	861 ± 154	498 ± 103	<0.001
ICAM-1 (ng/mL)	401 ± 82	248 ± 57	<0.001
Flow-Mediated Dilatation (%)	5.4 ± 1.6	10.3 ± 2.1	<0.001

Observation

Compared with normotensive controls, patients with essential hypertension demonstrated marked impairment of endothelial function. Nitric oxide bioavailability and flow-mediated dilatation were significantly reduced, whereas circulating endothelin-1, VCAM-1, and ICAM-1 concentrations were markedly increased. These findings are consistent with widespread endothelial activation and vascular dysfunction.

Inflammatory Biomarkers

Table 5: Inflammatory Biomarkers

Biomarker	Cases	Controls	p-value
hs-CRP (mg/L)	4.92 ± 1.84	1.38 ± 0.69	<0.001
IL-6 (pg/mL)	8.74 ± 2.31	3.26 ± 1.09	<0.001
TNF-α (pg/mL)	17.5 ± 4.2	9.1 ± 2.4	<0.001
MCP-1 (pg/mL)	318 ± 61	188 ± 47	<0.001

Observation: Hypertensive participants exhibited significantly elevated concentrations of all measured inflammatory biomarkers, indicating persistent low-grade systemic inflammation. Among these, IL-6 and hs-CRP showed the greatest relative increase compared with the control group.

Gut Dysbiosis Markers

Table 6: Gut Microbiome and Intestinal Permeability Markers

Variable	Cases	Controls	p-value
Firmicutes/Bacteroidetes Ratio	2.74 ± 0.63	1.46 ± 0.39	<0.001
Lactobacillus Abundance (log copies/g)	5.82 ± 0.76	7.14 ± 0.82	<0.001
Bifidobacterium Abundance (log copies/g)	6.04 ± 0.71	7.46 ± 0.78	<0.001
Escherichia coli Abundance (log copies/g)	6.89 ± 0.81	5.98 ± 0.69	<0.001
Serum LPS (EU/mL)	0.88 ± 0.22	0.42 ± 0.14	<0.001
Fecal Calprotectin (µg/g)	74.8 ± 18.5	34.6 ± 11.2	<0.001

Observation

Patients with essential hypertension showed clear evidence of gut microbial dysbiosis.

The Firmicutes/Bacteroidetes ratio was significantly increased, while beneficial bacterial genera such as Lactobacillus and Bifidobacterium were markedly depleted.

Increased Escherichia coli abundance, elevated serum lipopolysaccharide, and higher fecal calprotectin concentrations suggested impaired intestinal barrier integrity and systemic endotoxemia.

Summary of Primary Findings: The principal findings of this analytical case–control study can be summarized as follows:

1. Baseline demographic characteristics were comparable between groups with respect to age and sex, while hypertensive participants had significantly higher body mass index, waist circumference, and prevalence of adverse lifestyle factors.
2. Patients with essential hypertension exhibited marked endothelial dysfunction, characterized by reduced nitric oxide bioavailability and impaired flow-mediated dilatation, accompanied by increased endothelin-1, VCAM-1, and ICAM-1 levels.
3. Chronic low-grade systemic inflammation was evident among hypertensive individuals, with significantly elevated hs-CRP, IL-6, TNF-α, and MCP-1 concentrations.
4. Gut microbiome analysis demonstrated substantial dysbiosis, including depletion of beneficial bacterial taxa, increased Firmicutes/Bacteroidetes ratio, elevated serum lipopolysaccharide, and increased fecal calprotectin, supporting disruption of intestinal barrier function.

Advanced Statistical Analysis

Correlation Analysis: To investigate the interrelationship between endothelial dysfunction, inflammatory activation, gut microbial alterations, and blood pressure, Pearson's correlation coefficients were calculated.

Table 7: Correlation Matrix between Blood Pressure and Major Biomarkers in Hypertensive Patients (n = 160)

Variable	SBP	DBP	NO	ET-1	hs-CRP	IL-6	TNF-α	LPS	FMD
SBP	1.00								
DBP	0.84*	1.00							
Nitric Oxide	−0.71*	−0.66*	1.00						
Endothelin-1	0.74*	0.68*	−0.69*	1.00					
hs-CRP	0.63*	0.58*	−0.55*	0.61*	1.00				
IL-6	0.69*	0.61*	−0.59*	0.66*	0.79*	1.00			
TNF-α	0.60*	0.54*	−0.52*	0.57*	0.74*	0.76*	1.00		
Serum LPS	0.72*	0.65*	−0.68*	0.70*	0.73*	0.78*	0.69*	1.00	
FMD	−0.76*	−0.70*	0.82*	−0.74*	−0.66*	−0.68*	−0.61*	−0.73*	1.00

***p < 0.001**

Observation

Systolic blood pressure demonstrated a strong inverse correlation with nitric oxide (r = −0.71) and flow-mediated dilatation (r = −0.76), indicating that worsening endothelial function was associated with increasing blood pressure.

Conversely, systolic blood pressure showed strong positive correlations with endothelin-1, serum lipopolysaccharide, IL-6, and hs-CRP, suggesting that vascular inflammation and microbial endotoxemia contribute significantly to hypertension severity.

Flow-mediated dilatation exhibited the strongest positive correlation with nitric oxide (r = 0.82), reinforcing the central role of endothelial nitric oxide bioavailability in vascular homeostasis.

Association between Gut Dysbiosis and Endothelial Dysfunction

Table 8: Correlation between Gut Microbiota Markers and Endothelial Biomarkers

Gut Biomarker	Nitric Oxide	ET-1	VCAM-1	ICAM-1	FMD
Firmicutes/Bacteroidetes Ratio	−0.62	0.67	0.59	0.55	−0.69
Lactobacillus Abundance	0.65	−0.56	−0.49	−0.46	0.63
Bifidobacterium Abundance	0.67	−0.59	−0.53	−0.48	0.66
Serum LPS	−0.68	0.70	0.65	0.63	−0.73
Fecal Calprotectin	−0.56	0.60	0.54	0.50	−0.58

All correlations p < 0.001

Observation

Higher serum lipopolysaccharide concentrations were strongly associated with impaired endothelial function. Beneficial bacterial genera (Lactobacillus and Bifidobacterium) showed positive associations with nitric oxide bioavailability and flow-mediated dilatation, whereas increased Firmicutes/Bacteroidetes ratio and fecal calprotectin correlated with endothelial injury and vascular inflammation. These findings support the proposed gut–vascular–immune axis in essential hypertension.

Multiple Linear Regression Analysis

Predictors of Systolic Blood Pressure: Variables showed significant univariate associations were entered into a multiple linear regression model.

Table 9: Multiple Linear Regression Analysis

Predictor	β Coefficient	Standard Error	Standardized β	p-value
Age	0.12	0.08	0.07	0.118
BMI	0.39	0.09	0.18	0.004
Nitric Oxide	−0.78	0.11	−0.36	<0.001
Endothelin-1	1.94	0.42	0.28	<0.001
IL-6	0.81	0.21	0.24	<0.001
Serum LPS	8.92	1.46	0.33	<0.001
hs-CRP	0.46	0.18	0.12	0.018

Model Statistics

- R^2 = **0.71**
- Adjusted R^2 = **0.69**
- F = **62.8**
- p < **0.001**

Observation:

Approximately 71% of the variability in systolic blood pressure was explained by the variables. Reduced nitric oxide emerged as the strongest negative predictor of systolic blood pressure, while elevated serum lipopolysaccharide and endothelin-1 were the strongest positive predictors, independent of age and body mass index.

Multivariable Logistic Regression: Independent predictors of essential hypertension were evaluated after adjustment for potential confounding variables.

Table 10: Multivariable Logistic Regression Analysis

Variable	Adjusted Odds Ratio	95% Confidence Interval	p-value
BMI	1.14	1.06–1.24	0.002
Smoking	1.72	1.03–2.89	0.039
Nitric Oxide (per 5 µmol/L decrease)	2.81	1.98–3.97	<0.001
Endothelin-1	2.54	1.71–3.76	<0.001
IL-6	1.68	1.29–2.19	<0.001
Serum LPS	3.32	2.11–5.23	<0.001
Firmicutes/Bacteroidetes Ratio	1.91	1.36–2.68	<0.001

Hosmer–Lemeshow Test, $\chi^2 = 5.81$, p = 0.667 (Model showed good calibration.)

Observation

Serum lipopolysaccharide exhibited the highest adjusted odds ratio among all evaluated biomarkers, suggesting that intestinal endotoxemia was the strongest independent predictor of essential hypertension within this study population. Reduced nitric oxide concentration, elevated endothelin-1, increased IL-6, and gut microbial dysbiosis also remained statistically significant independent predictors after adjustment for conventional cardiovascular risk factors.

Receiver Operating Characteristic (ROC) Curve Analysis: Receiver operating characteristic analysis was performed to assess the diagnostic performance of endothelial, inflammatory, and microbial biomarkers.

Table 11: ROC Analysis of Individual Biomarkers

Biomarker	AUC	Sensitivity (%)	Specificity (%)
Nitric Oxide	0.901	85.6	84.4
Endothelin-1	0.916	87.5	86.3
IL-6	0.892	84.4	83.8
hs-CRP	0.857	80.6	79.4
Serum LPS	0.934	89.4	88.8
Flow-Mediated Dilatation	0.928	88.8	87.5

Combined Biomarker Model: A predictive model combining:

- Nitric Oxide
- Endothelin-1
- IL-6
- Serum Lipopolysaccharide
- Flow-Mediated Dilatation

Was subsequently constructed.

Table 12: ROC Analysis of Combined Biomarker Panel

Model	AUC	Sensitivity	Specificity
Combined Biomarker Panel	**0.972**	**94.4%**	**92.5%**

Observation

The combined biomarker panel showed excellent discriminatory ability for differentiating hypertensive patients from normotensive controls, with an AUC of 0.972, substantially outperforming individual biomarkers. This finding suggests that integrating endothelial, inflammatory, and gut microbiota-derived markers may enhance risk stratification and early detection of essential hypertension.

Subgroup Analysis According to Hypertension Severity: Among the 160 hypertensive participants:

- Stage I hypertension: 66 (41.3%)
- Stage II hypertension: 94 (58.7%)

Progressive worsening of endothelial dysfunction, inflammatory activation, and gut dysbiosis was observed with increasing hypertension severity.

Patients with Stage II hypertension demonstrated significantly lower nitric oxide levels, reduced flow-mediated dilatation, higher endothelin-1, increased inflammatory cytokines, elevated serum lipopolysaccharide, and a greater Firmicutes/Bacteroidetes ratio than those with Stage I disease (all $p < 0.001$).

Discussion

Overview

The present hospital-based analytical case–control study was designed to comprehensively evaluate the interrelationship between endothelial dysfunction, gut microbial dysbiosis, chronic systemic inflammation, and essential hypertension [23]. By integrating vascular physiology, inflammatory biomarker profiling, and microbiome assessment within a single analytical framework, the study explored the concept of a gut–vascular–immune axis in the pathogenesis of hypertension [24].

The principal findings showed that patients with essential hypertension exhibited significantly impaired endothelial function, characterized by reduced nitric oxide bioavailability and diminished flow-mediated dilatation, together with increased circulating endothelin-1, VCAM-1, and ICAM-1 concentrations. These vascular abnormalities coexisted with marked elevations in inflammatory biomarkers (hs-CRP, IL-6, TNF-α, and MCP-1) and evidence of gut microbial dysbiosis, including depletion of beneficial bacterial taxa, increased Firmicutes/Bacteroidetes ratio, elevated serum lipopolysaccharide, and increased fecal calprotectin [25]. Multivariable analyses further indicated that serum lipopolysaccharide, nitric oxide depletion, endothelin-1, and IL-6 were independent determinants of hypertension, supporting the hypothesis that endothelial injury, inflammation, and intestinal microbial imbalance interact synergistically in disease pathogenesis [26].

Demographic and Clinical Characteristics: The demographic characteristics of the case and control groups were comparable with respect to age and sex, reducing the likelihood that these variables substantially influenced the observed biological differences [27]. In contrast, body mass index, waist circumference, sedentary behavior, smoking, and family history of hypertension were significantly more common among hypertensive participants. These observations are consistent with the well-established contribution of obesity, central adiposity, unhealthy lifestyle, and genetic susceptibility to cardiovascular risk [28].

Central obesity is increasingly recognized as a metabolically active state that promotes chronic inflammation through adipose tissue-derived cytokines and adipokines. Excess visceral fat contributes to insulin resistance, oxidative stress, endothelial dysfunction, and activation of the renin–angiotensin–aldosterone system [29]. The higher prevalence of sedentary behavior observed among hypertensive participants may further exacerbate these mechanisms by reducing endothelial nitric oxide synthase activity, impairing vascular compliance, and diminishing anti-inflammatory responses associated with regular physical activity [30].

Endothelial Dysfunction as a Central Pathogenic Mechanism: One of the most striking findings of

this study was the profound impairment of endothelial function among patients with essential hypertension. Reduced serum nitric oxide concentrations and significantly diminished flow-mediated dilatation were accompanied by marked elevations in endothelin-1, VCAM-1, and ICAM-1. Together, these observations indicate widespread endothelial activation and dysfunction [31].

The endothelium serves as a dynamic endocrine organ that regulates vascular tone, coagulation, inflammation, permeability, and smooth muscle proliferation. Nitric oxide is the principal vasodilator responsible for maintaining vascular homeostasis [35]. Decreased nitric oxide bioavailability in hypertensive patients may reflect enhanced oxidative degradation, reduced endothelial nitric oxide synthase activity, or enzymatic uncoupling secondary to chronic inflammation [32]. The observed inverse correlation between nitric oxide and systolic blood pressure supports its central role in preserving vascular relaxation and limiting peripheral vascular resistance.

Elevated endothelin-1 concentrations further suggest an imbalance between vasodilatory and vasoconstrictive pathways. Endothelin-1 not only induces sustained vasoconstriction but also promotes oxidative stress, vascular smooth muscle hypertrophy, extracellular matrix deposition, and arterial fibrosis. Increased VCAM-1 and ICAM-1 levels indicate activation of endothelial inflammatory pathways that facilitate leukocyte adhesion and transendothelial migration, thereby perpetuating vascular injury [33].

Collectively, these findings reinforce the concept that endothelial dysfunction is not merely a consequence of elevated blood pressure but rather an early pathogenic event that contributes to disease initiation and progression.

Systemic Inflammation and Hypertension: The present study demonstrated significantly elevated circulating concentrations of hs-CRP, IL-6, TNF-α, and MCP-1 among hypertensive participants, indicating the presence of chronic low-grade systemic inflammation.

Interleukin-6 occupies a pivotal position within inflammatory signaling by stimulating hepatic synthesis of C-reactive protein, promoting leukocyte activation, and amplifying endothelial oxidative stress. The strong positive correlation between IL-6 and systolic blood pressure observed in the present study suggests that inflammatory activation may directly influence vascular resistance [34]. Tumor necrosis factor-alpha further contributes by suppressing endothelial nitric oxide synthase expression, increasing reactive oxygen species generation, and promoting endothelial apoptosis. MCP-1 facilitates recruitment of circulating monocytes into the vascular wall, accelerating chronic vascular inflammation and remodeling.

High-sensitivity C-reactive protein has traditionally been considered a marker of inflammation; however, accumulating evidence suggests that CRP itself may impair endothelial nitric oxide production, enhance endothelin-1 synthesis, and promote adhesion molecule expression. Thus, the elevated hs-CRP concentrations observed in hypertensive participants may reflect both systemic inflammation and active participation in vascular injury [35].

The concurrent elevation of these inflammatory mediators supports the view that essential hypertension is characterized by persistent immune activation rather than isolated hemodynamic dysregulation.

Gut Dysbiosis and the Gut–Vascular–Immune Axis: Perhaps the most novel aspect of this study was the simultaneous evaluation of gut microbial composition and vascular biomarkers. Hypertensive patients showed a significantly increased Firmicutes/Bacteroidetes ratio, reduced abundance of Lactobacillus and Bifidobacterium, increased Escherichia coli abundance, elevated serum lipopolysaccharide, and higher fecal calprotectin concentrations. These findings indicate disruption of intestinal microbial homeostasis together with increased intestinal permeability.

Beneficial commensal bacteria contribute to host health by producing short-chain fatty acids, maintaining epithelial tight junctions, regulating immune tolerance, and supporting endothelial function. Their depletion may reduce production of butyrate, propionate, and acetate, thereby diminishing anti-inflammatory signaling and nitric oxide bioavailability. Conversely, expansion of potentially pathogenic bacteria may enhance production of endotoxins and inflammatory metabolites. The observed increase in serum lipopolysaccharide is particularly important because it suggests translocation of bacterial products into the systemic circulation. Lipopolysaccharide activates Toll-like receptor-4 signaling on endothelial and immune cells, triggering activation of NF-κB and downstream production of IL-6, TNF-α, and other inflammatory mediators. This sequence provides a biologically plausible mechanism linking intestinal barrier dysfunction to endothelial injury and hypertension.

The positive correlation between serum lipopolysaccharide and endothelin-1, together with its inverse association with nitric oxide and flow-mediated dilatation, further supports the hypothesis

that microbial endotoxemia contributes directly to vascular dysfunction.

Interaction between Endothelial Dysfunction and Inflammation: The study findings indicate that endothelial dysfunction and inflammation are closely interconnected rather than independent processes. Elevated inflammatory cytokines promote oxidative degradation of nitric oxide and increase endothelin-1 production, while endothelial activation enhances leukocyte recruitment through increased expression of VCAM-1 and ICAM-1. This reciprocal relationship establishes a self-perpetuating cycle of vascular injury.

Reactive oxygen species generated during chronic inflammation rapidly combine with nitric oxide to form peroxynitrite, thereby reducing nitric oxide bioavailability and amplifying endothelial dysfunction. Simultaneously, inflammatory cytokines activate vascular smooth muscle cells, promote collagen deposition, and accelerate arterial stiffening. The observed associations between inflammatory biomarkers and impaired flow-mediated dilatation in the present study are consistent with these mechanisms.

Clinical Implications of the Multivariable Analyses: Multivariable regression analyses identified serum lipopolysaccharide, reduced nitric oxide, elevated endothelin-1, IL-6, and gut microbial dysbiosis as independent predictors of essential hypertension, even after adjustment for age, body mass index, smoking, and other conventional risk factors. These observations suggest that microbial and endothelial biomarkers provide information beyond traditional cardiovascular risk assessment. The combined biomarker model achieved excellent diagnostic discrimination, outperforming individual biomarkers. This finding supports the concept that hypertension arises from multiple converging biological pathways and may therefore be more accurately characterized using integrated biomarker profiling rather than isolated laboratory parameters.

Although further validation in prospective cohorts would be necessary, these findings raise the possibility that future risk prediction models could incorporate endothelial function, inflammatory mediators, and microbiome-derived biomarkers to improve early identification of individuals at increased cardiovascular risk.

Potential Therapeutic Implications: The integrated findings of this study have several potential therapeutic implications. Strategies aimed at restoring endothelial nitric oxide bioavailability, reducing oxidative stress, and suppressing chronic inflammation may complement conventional blood pressure-lowering therapies.

Equally important is the possibility that modulation of the gut microbiome may represent a novel adjunctive approach in hypertension management. Dietary interventions rich in fermentable fiber, increased consumption of plant-based foods, and targeted microbiome-modulating strategies have the potential to enhance short-chain fatty acid production, improve intestinal barrier integrity, and attenuate systemic inflammation. While the present study was not designed to evaluate therapeutic interventions, the observed associations provide a mechanistic rationale for future interventional trials targeting the gut–vascular–immune axis.

Future Research Directions: The findings of this study provide several avenues for future investigation. Prospective longitudinal studies should evaluate whether microbial dysbiosis and endothelial dysfunction predict the development of hypertension in initially normotensive individuals.

Randomized controlled trials examining microbiome-directed interventions, including dietary modification and targeted microbial therapies, may clarify whether restoration of microbial homeostasis improves endothelial function and blood pressure control.

Integration of multi-omics approaches, including metagenomics, metabolomics, transcriptomics, and proteomics, may further elucidate the molecular mechanisms linking the intestinal microbiome with vascular biology. Such approaches could facilitate the identification of novel therapeutic targets and personalized strategies for cardiovascular prevention.

Conclusion

This analytical case–control study illustrates a multidimensional model of essential hypertension in which endothelial dysfunction, chronic systemic inflammation, and gut microbial dysbiosis appear to be closely interconnected.

Within this conceptual framework, individuals with essential hypertension showed impaired endothelial function characterized by reduced nitric oxide bioavailability and diminished flow-mediated dilatation, together with increased endothelin-1 and endothelial adhesion molecules. These vascular changes coexisted with elevated inflammatory mediators and markers of intestinal barrier dysfunction, supporting the concept of a coordinated gut–vascular–immune axis.

The analyses further suggest that reduced nitric oxide, elevated endothelin-1, increased interleukin-6, and circulating lipopolysaccharide may each contribute independently to hypertension risk, while a combined biomarker approach could potentially provide greater discriminatory value than any single marker alone. Overall, the study

highlights the importance of viewing essential hypertension not solely as a disorder of elevated arterial pressure but as a systemic condition involving vascular, immunological, metabolic, and microbial interactions.

Future longitudinal and interventional investigations will be required to determine whether modification of these pathways can improve blood pressure control and reduce long-term cardiovascular complications.

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