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ORIGINAL ARTICLE

Association between Intestinal *Escherichia coli* Colonization and Endothelial Dysfunction Biomarkers in Apparently Healthy Adults

Bayda Thamr Hameed*
Department of Medical Laboratory Techniques, Kirkuk Health and Medical Technical College, Northern Technical University, Kirkuk, Iraq

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ABSTRACT

The role of microbiome changes as potential contributors of systemic inflammation and oxidative stress has become increasingly apparent. One Gram-negative bacterium found in the intestines is *Escherichia coli. E. coli* colonization may also be linked to elevated lipopolysaccharide levels, which cause endothelial dysfunction. via inflammation and oxidative mechanisms. It is unclear whether there is any link between colonization by intestinal *E. coli* and endothelial dysfunction in healthy individuals. The current study attempted to examine the relationship between the presence of intestinal colonization of *E. coli* with the biomarkers of endothelial dysfunction in apparently healthy adults, as well as the possible role played by endotoxemia, inflammation, oxidative stress, and metabolism alteration in this correlation. A cross-sectional study was carried out among 150 apparently healthy individuals aged 20–60 years old in Kirkuk Province, Iraq. The participants were divided into a control group (n = 75) and *E. coli* colonized group (n = 75) according to their culture test results of stool samples. VCAM-1, ICAM-1, ET-1, NO, LPS, hs-CRP, TNF-α, IL-6, MDA, TAC, lipid profile parameters, fasting blood glucose level, insulin level, and HOMA-IR were examined in serum. Correlation and multiple regression analysis were employed in order to determine the factors related to endothelial dysfunction. It was revealed that serum levels of VCAM-1, ICAM-1, ET-1, LPS, hs-CRP, TNF-α, IL-6, MDA, total cholesterol, triglycerides, LDL-C, fasting glucose, insulin, and HOMA-IR were higher significantly in cases of *E. coli* colonization in the intestines than in the control group ($P < 0.05$). On the other hand, NO, TAC, and HDL-C levels in the group with colonization were significantly lower. The concentrations of serum LPS had positive correlations significantly with VCAM-1 ($r = 0.62$), ICAM-1 ($r = 0.58$), TNF-α ($r = 0.66$), IL-6 ($r = 0.61$), and MDA ($r = 0.55$) whereas there was a significant negative correlation between serum LPS and NO ($r = -0.59$) ($P < 0.001$). It was revealed by multiple regression analysis that the serum LPS and TNF-α were independent predictors for endothelial dysfunction. These findings suggest an association between intestinal *E. coli* colonization and endothelial dysfunction. However, because of the cross-sectional design, no causal relationship can be established.

Corresponding Author:

* Bayda Thamr Hameed
Department of Medical Laboratory Techniques, Kirkuk Health and Medical Technical College, Northern Technical University, Kirkuk, Iraq
Email: Baydathamr@ntu.edu.iq

1- INTRODUCTION

The gastrointestinal tract of humans is an intricate microbial habitat that acts an important part in keeping the host healthy, metabolically efficient, immunologically regulated, and maintains the balance of intestine. In a physiological state, the intestinal flora is responsible for the process of nutrition metabolism, acts against pathogens, and regulates the immune response [1]. However, changes in the composition of the microbial community or increased population of some bacteria can lead to intestinal imbalance and inflammation [2]. *Escherichia coli* represents the most frequent Gram-negative organism colonizing the human gut [3]. While many are classified as commensal organisms, their colonization and overgrowth can lead to the increased shedding of bacterial components, specifically lipopolysaccharides (LPS), which are one of the main components of the outer membrane of Gram-negative bacteria [4]. Increased levels of LPS have been linked to the development of increased gut permeability, endotoxemia, inflammation, and vascular dysfunction [5]. Endothelial dysfunction has been well acknowledged to be one of the earliest events involved in the development of cardiovascular diseases. Endothelium plays a vital role in controlling the vascular tone, inflammation, coagulation, and cellular adhesion processes [6]. An endothelial dysfunction entails diminished nitric oxide availability, up-regulated expression of adhesion molecules like VCAM-1 (vascular cell adhesion molecule-1) and ICAM-1 (intercellular adhesion molecule-1) and enhanced production of vasoconstrictors like endothelin-1 [7]. This causes vascular inflammation and initiates and promotes atherosclerosis and other cardiovascular diseases [8].

There is increasing evidence that endotoxins derived from the gut contribute to changes in the physiology of the endothelium via chronic inflammation and oxidative stress [9]. Lipopolysaccharides activate TLR4 signaling pathways, resulting in the release of pro-inflammatory cytokines. the secretion of pro-inflammatory cytokines, such as TNF-α and IL-6 [10]. Such inflammatory factors can reduce the formation of nitric oxide by the endothelium, increase oxidative stress, and induce endothelial adhesion molecule expression [11]. Persistent endotoxemia therefore represents a possible mechanism of connection between the change in microbiota and cardiovascular risks [12]. Many studies have examined the link between dysbiosis in the gut microbiome and metabolic or cardiovascular disease; but there are few data on the link between *E. coli* colonisation in the intestine and endothelial dysfunction in apparently healthy individuals [13]. Learning about such a link may help understand the vascular effects of bacteria derived from the gut before any disease develops [14]. In this respect, this study was aimed at exploring the association between colonization of *Escherichia coli* bacteria in the intestines and biomarkers of endothelial dysfunction among healthy individuals. Furthermore, serum lipopolysaccharide levels, inflammation markers, oxidative stress biomarkers, lipid profile, and glycaemia markers were also assessed in this study to understand the possible mechanisms involved.

2- MATERIALS AND METHODS

2.1 Study Design

The cross-sectional case control study was done from January to September 2026 in Kirkuk Province, Iraq. It was designed to determine the link between intestinal *Escherichia coli* colonization and biomarkers for endothelial dysfunction among apparently healthy adults. Because of the cross-sectional design, causal relationships could not be established.

2.2 Study Population

A total number of 150 individuals considered to be healthy were involved in this cross-sectional study. Subjects were categorized into two categories depending on the microbiological culture test results of the stools performed in accordance with the procedure of the study. The first category comprised 75 subjects having *Escherichia coli* colonizing their intestine, while the second one was made up of 75 non-colonized subjects with respect to *Escherichia coli*. In order to make sure that the results are reliable and any confounders are avoided, only healthy people aged between 20-60 who did not have antibiotics for three months before and who volunteered to be part of the study were selected as the sample group. Those having a history of diabetes, hypertension, cardiovascular problems, chronic kidney disease, chronic liver disease, autoimmune diseases, and other infections were not included in the sample. Smokers, pregnant women, and those under anti-inflammatory and immunosuppressive drugs were excluded because these factors affect the inflammation process and the microbiome in the gut.

2.3 Sample Size Calculation

The sample size calculation was performed using the G*Power software (version 3.1.9.7*).* Two-tailed independent samples t-test was chosen with a significance level (α) of 0.05, a power of the test (1−β) equal to 0.80 and a high effect size (Cohen's d=0.80), considering the previously reported studies that evaluated the relationship between biomarkers of endothelial dysfunction and intestinal microbiota. A minimum sample size of 52 participants in each group (104 participants in total) was calculated. In order to increase the power of the study, 75 participants were recruited in each group (150 participants in total).

2.4 Collection and Processing of Stool Samples

New samples of feces were collected from each participant in a sterile and waterproof container using aseptic technique. The collected samples were then sent immediately to the microbiology laboratory where they were tested on the same day to guarantee the viability of bacteria and the validity of microbiological tests. The bacteria were isolated using 1 gram of each stool sample that was suspended in sterile normal saline solution and then subjected to ten-fold dilution series. Selected dilutions were plated onto differential and selective culture media such as MacConkey agar and eosin methylene blue (EMB) agar for the isolation and tentative identification of Escherichia coli. These plates were then incubated at 37°C for 24 hours under aerobic conditions. The results after incubation were based on characteristics and lactose fermentation of the bacteria.

2.5 Identification of *Escherichia coli*

The presumptive isolates of *E. coli* were selected by taking into consideration the morphological characteristics of their colonies and some biochemical properties. Isolates having the morphological features of lactose fermenter on MacConkey agar plate and the metallic green coloration on Eosin Methylene Blue agar plate were chosen for further confirmation. The selected isolates were then put through some standard microbiological tests such as Gram stain test, indole test, Methyl Red (MR) test, Voges-Proskauer (VP) test, citrate test, urea test, and TSI (Triple sugar Iron) test. The purpose of the Gram stain test was to ascertain that the isolates were Gram negative bacilli. Isolates which show the biochemical characteristics of *E. coli*, such as Indole-positive, Methyl red-positive and negative results for the Voges-Proskauer test, citrate utilization, urease test and also having the correct TSI test results, were identified as *Escherichia coli* and were used in further experiments.

2.6 Blood Sample Collection

Blood samples, collected after 10 to 12 hours of overnight fasting, were obtained from each individual using aseptic techniques by drawing 5 mL of venous blood from each individual using sterile disposable syringes.
The collected blood samples were put in plain tubes and left to clot at room temperature for a suitable amount of time. Centrifugation was performed on these samples for 10 minutes at 3000 rpm, and serum was separated from the cellular fraction. Serum samples were then divided in sterile microcentrifuge tubes to avoid repetitive freezing and thawing and stored at −80°C until further analysis. This method was used to ensure the stability of the biomarkers during the study period.

2.7 Biochemical Measurements

2.7.1 Endothelial Dysfunction Biomarkers

Biomarkers that indicate endothelial dysfunction are routinely used for the assessment of vascular health and the study of endothelial function. Thus, a number of such biomarkers was examined in the serum samples obtained from all volunteers in accordance with manufacturers' protocols with the use of commercially available ELISA kits. Levels of Vascular Cell Adhesion Molecule-1 (VCAM-1) were quantified by means of ELISA. VCAM-1 is one of the key endothelial adhesion molecules, which participates in leukocyte recruitment and vascular inflammation; increased levels of VCAM-1 are associated with endothelial dysfunction. The concentration of Intercellular Adhesion Molecule-1 (ICAM-1) was also measured via sandwich ELISA method. ICAM-1 is considered to be an important mediator of leukocytes adhesion and migration through the vascular endothelium and serves as a marker of the endothelial damage and inflammation. Endothelin-1 (ET-1), which is a potent vasoactive peptide synthesized by endothelial cells, was quantitated via human ELISA kit. Higher blood level of endothelin-1 is considered to be an indicator of impaired endothelial function and cardiovascular disease. Bioavailability of nitric oxide (NO) was estimated indirectly based on serum concentration of nitrate/nitrite, its stable oxidation products, using the Griess assay. Nitric oxide (NO) plays an important role in regulating vascular tone and endothelial cell function and lack of availability of nitric oxide is one of the indicators of endothelial dysfunction. All absorbance

values were determined by a microplate reader at the wavelengths recommended by the assay manufacturer and the concentration of the biomarker was quantitated by a standard curve for each assay.

2.7.2 Inflammatory Biomarkers

In order to determine the inflammatory state of the study subjects and its possible link with *Escherichia coli* infection in the gut and dysfunction of endothelium, levels of certain inflammatory markers were analyzed in blood serum. Determination of the concentration of the markers was done by means of commercially available ELISA kits as per manufacturers' specifications. Measurement of hs-CRP was done using a high sensitivity ELISA test. High-Sensitivity C-Reactive Protein (hs-CRP) is a known indicator of systemic inflammation and has been found to be strongly related to endothelial dysfunction. Quantification of Tumor Necrosis Factor-Alpha (TNF-α) was done by employing the use of an ELISA kit specific to humans. TNF-α is a pro-inflammatory cytokine whose major functions include immune activation, inflammation, and vascular endothelial damage. The levels of interleukin-6 (IL-6) were quantified by the means of sandwich ELISA. Interleukin-6 (IL-6) is a multi-functional cytokine that plays a regulatory role in inflammatory processes and serves as a mediator between chronic inflammation and endothelial dysfunction. All assays were carried out according to the instructions of the manufacturers, and the absorbance values were recorded in the microplate reader. The concentration of hs-CRP, TNF-α, and IL-6 was determined from the standard curve prepared for each particular assay and was given in the units recommended by the kit manufacturers.

2.7.3 Assessment of Endotoxemia

The concentration of serum lipopolysaccharide was used to assess the status of endotoxemia and its intensity in the study participants. The lipopolysaccharide molecule is the main component of the outer membrane of Gram-negative bacteria such as E. coli, and increased levels in serum were seen as the evidence of increased permeability of the intestine and transfer of bacteria from the intestinal lumen into blood. Serum LPS concentrations were determined using a chromogenic Limulus Amebocyte The test of Lysate (LAL) is performed according to the instructions of the manufacturer. The LAL test works due to the colorimetric response that occurs when endotoxins activate Limulus amebocyte lysate, which is proportional to the amount of endotoxins in the solution. The absorbance is determined using the microplate reader, and the concentration of endotoxins is calculated by the standard curve. The results were reported in terms of endotoxin units per milliliter (EU/mL). Higher values represent larger quantities of endotoxins in the system, thus, an increased inflammatory response due to intestinal bacterial colonization.

2.7.4 Oxidative Stress Parameters

Malondialdehyde (MDA) was estimated by measuring the amount of TBARS in the presence of malondialdehyde using thiobarbituric acid reactive substances (TBARS), which are markers for lipid peroxidation and oxidative stress. High levels of MDA are an indication of high oxidative stress. Total Antioxidant Capacity (TAC) was assessed by means of the colorimetric total antioxidant assay using the commercially available kit to determine the antioxidant defense status of the serum.

2.7.5 Lipid Profile Analysis

Serum levels of lipid profile parameters were estimated by automated biochemical analyzers according to standard protocols in the lab. Lipid profile analysis was performed for total cholesterol (TC), triglycerides (TG), HDL cholesterol (HDL-C), and LDL cholesterol (LDL-C). These are important markers of cardiovascular disease and metabolic disorders. LDL-C levels were calculated using the Friedewald equation, as shown: (LDL- C = TC – HDL - C – TG / 5). The above formula was used when triglyceride concentration was within the range that made the calculation possible. Lipid parameters derived were used to measure dyslipidemia and its possible relationship to *Escherichia coli* bacteria colonization of the intestines and endothelial dysfunction indicators.

2.7.6 Glycemic Parameters

Blood Glucose Levels were analyzed using a colorimetric method based on enzymes. Serum concentration of insulin was measured using commercial ELISA Kits. The calculation of insulin resistance was made using the HOMA-IR model. The formula is provided: (HOMA – IR = Fasting Insulin (mU/mL) * Fasting glucose times (mg/dL) / 405. This index was used to estimate the degree of insulin resistance and its relation with the presence of intestinal *E. coli* and endothelial dysfunction.

2.8 Anthropometric Measurements

Height and body weight were determined by applying standard measurement techniques on participants dressed lightly but not wearing any footwear. The body mass index (BMI) was computed as a measure of adiposity using the formula: (BMI = Weight (kg)/ Height2 (m2). Moreover, waist circumference was measured using a non-elastic measuring tape in the mid-point between the lower rib margin and the iliac crest. This measure was used to identify central obesity that is closely associated with metabolic risks and endothelial dysfunction.

2.9 Statistical Analysis

The statistical analyses were carried out by SPSS version 26. The continuous data were expressed as Mean±SD. The comparisons between groups were performed using t-test, Mann-Whitney U-test, and chi-square test. For the relationship analysis, Pearson correlation and multiple regression were utilized. $P<0.05$ was statistically significant.

3- RESULTS AND DISCUSSION

3.1 Characteristics of the Study Population

All together 150 seemingly healthy subjects were recruited for the experiment. Seventy-five of those subjects were found to be colonized with *Escherichia coli* from intestine, while the other seventy-five were determined to be non-colonized. From Table 1, it can be noted that there were no statistically significant differences between the two groups in terms of age, body mass index (BMI), and waist circumference ($P > 0.05$).

Table (1): Demographic and anthropometric characteristics of the study population

Parameter	Control (n=75)	*E. coli* Colonized (n=75)	P-value
Age (years)	38.4 ± 9.2	39.1 ± 8.7	0.64
BMI (kg/m²)	26.1 ± 3.5	26.8 ± 3.8	0.28
Waist Circumference (cm)	89.7 ± 8.9	92.4 ± 9.3	0.08

3.2 Endothelial Dysfunction Biomarkers

Differences in serum biomarkers of endothelial dysfunction were found between the two groups under study. The colonized subjects had significantly higher levels of serum VCAM-1, ICAM-1, and endothelin-1 compared to those of the non-colonized subjects ($P<0.001$). However, significant lower level of serum nitric-oxide (NO) was observed in the colonized group. As depicted in Table 2 and Figure 1 below, the VCAM-1 increased by 33.6%, while the concentration of ICAM-1 increased by 44.4% among those that were colonized. The concentration of endothelin-1 was 60% higher, while NO availability was 32% lower.

Table (2): Endothelial dysfunction biomarkers in study groups

Biomarker	Control	Colonized	P-value
VCAM-1 (ng/mL)	512 ± 78	684 ± 96	<0.001
ICAM-1 (ng/mL)	198 ± 34	286 ± 45	<0.001
Endothelin-1 (pg/mL)	2.8 ± 0.7	4.5 ± 1.1	<0.001
NO (µmol/L)	35.6 ± 6.8	24.3 ± 5.9	<0.001

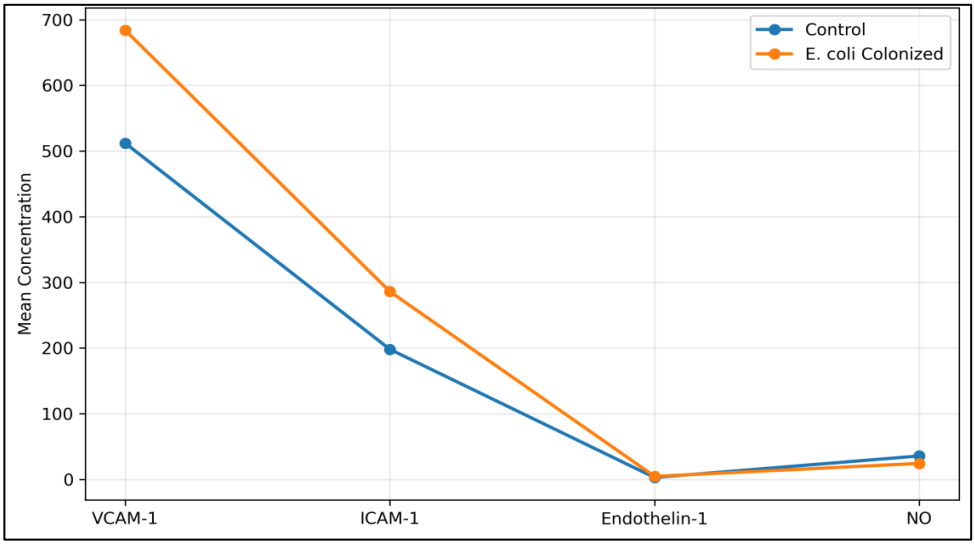


Fig (1): Comparison of endothelial dysfunction biomarkers between control and colonized groups

3.3 Inflammatory Biomarkers

The colonized group showed markedly elevated levels of systemic inflammatory markers compared to controls (see Table 3). Concentrations of serum hs-CRP were seen to rise by more than twofold in the colonized population while TNF-α and IL-6 rose by about 71% and 102%, respectively (P<0.001). These findings, presented graphically in Figure 2, indicate a strong inflammatory response associated with intestinal *E. coli* colonization.

Table (3): Inflammatory biomarkers among study groups

Biomarker	Control	Colonized	P-value
hs-CRP (mg/L)	1.9 ± 0.8	4.2 ± 1.3	<0.001
TNF-α (pg/mL)	12.5 ± 3.2	21.4 ± 4.8	<0.001
IL-6 (pg/mL)	4.8 ± 1.4	9.7 ± 2.3	<0.001

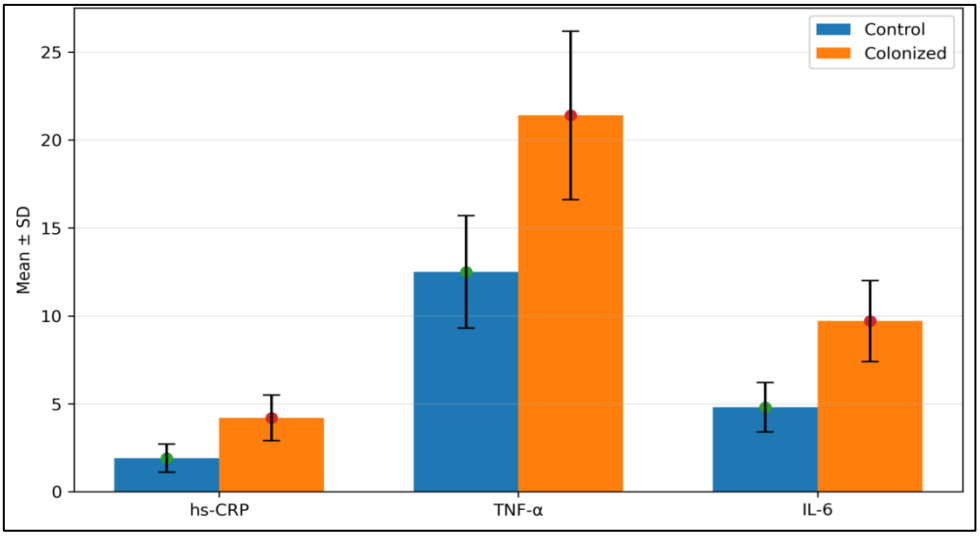


Fig (2): Serum inflammatory biomarker levels in control and colonized participants

3.4 Serum Lipopolysaccharide (LPS) Concentrations

As depicted in Table 4, the serum LPS levels were significantly elevated in colonized individuals as compared to control individuals (0.63 ± 0.17 vs. 0.21 ± 0.08 EU/mL; P < 0.001). This increase in serum levels of endotoxins implies increased translocation of bacteria products in the bloodstream, which might be indicative of metabolic endotoxemia.

Table (4): Serum LPS concentrations in study groups

Parameter	Control	Colonized	P-value
LPS (EU/mL)	0.21 ± 0.08	0.63 ± 0.17	<0.001

3.5 Oxidative Stress Parameters

Changes in oxidative stress parameters were statistically significant in colonized participants (Table 5). MDA levels were found to be significantly high and TAC levels to be significantly low compared to control group (P < 0.001). It was seen from figure 4 that MDA levels were increased by about 76% and TAC levels were decreased by about 32%.

Table (5): Oxidative stress biomarkers in study groups

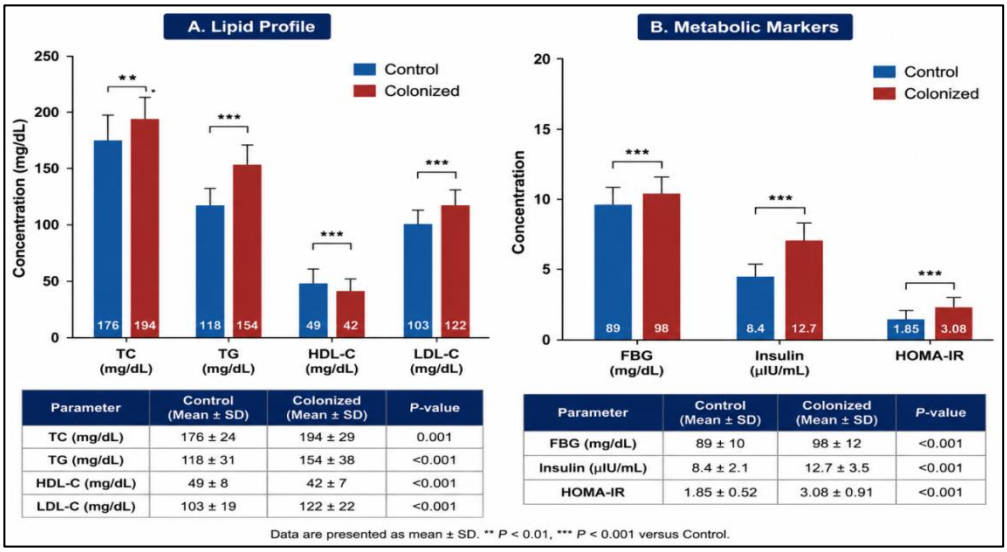
Parameter	Control	Colonized	P-value
MDA (nmol/mL)	2.9 ± 0.6	5.1 ± 0.9	<0.001
TAC (mmol/L)	1.42 ± 0.23	0.96 ± 0.18	<0.001

3.6 Lipid Profile and Glycemic Parameters

Differences in metabolic parameters between the two groups were found to be statistically significant (Table 6). The colonized group showed increased total cholesterol, triglycerides, LDL-C, fasting blood glucose, fasting insulin, and HOMA-IR levels compared to decreased levels of HDL-C (P<0.01). As is evident from Figure 3, the rise in triglyceride level was 31%, 18% in LDL-C, 51% in fasting insulin, and 66% in HOMA-IR, suggesting that there were metabolic abnormalities with decreased sensitivity to insulin among those who are colonized.

Table (6): Lipid profile and glycemic parameters among study groups

Parameter	Control	Colonized	P-value
TC (mg/dL)	176 ± 24	194 ± 29	0.001
TG (mg/dL)	118 ± 31	154 ± 38	<0.001
HDL-C (mg/dL)	49 ± 8	42 ± 7	<0.001
LDL-C (mg/dL)	103 ± 19	122 ± 22	<0.001
FBG (mg/dL)	89 ± 10	98 ± 12	<0.001
Insulin (µIU/mL)	8.4 ± 2.1	12.7 ± 3.5	<0.001
HOMA-IR	1.85 ± 0.52	3.08 ± 0.91	<0.001



Parameter	Control (Mean ± SD)	Colonized (Mean ± SD)	P-value
TC (mg/dL)	176 ± 24	194 ± 29	0.001
TG (mg/dL)	118 ± 31	154 ± 38	<0.001
HDL-C (mg/dL)	49 ± 8	42 ± 7	<0.001
LDL-C (mg/dL)	103 ± 19	122 ± 22	<0.001

Parameter	Control (Mean ± SD)	Colonized (Mean ± SD)	P-value
FBG (mg/dL)	89 ± 10	98 ± 12	<0.001
Insulin (µIU/mL)	8.4 ± 2.1	12.7 ± 3.5	<0.001
HOMA-IR	1.85 ± 0.52	3.08 ± 0.91	<0.001

Data are presented as mean ± SD. ** $P < 0.01$, *** $P < 0.001$ versus Control.

Fig (3): Comparison of metabolic parameters between study groups

3.7 Correlation Analysis

The results from the Pearson correlation test indicated that there were significant correlations between LPS level in the blood and indicators of endothelial dysfunction, inflammation, and oxidative stress (Table 7). The results indicated that there were positive correlations between LPS and VCAM-1 (r= 0.62), ICAM-1 (r= 0.58), TNF-α (r= 0.66), IL-6 (r= 0.61), and MDA (r= 0.55), while LPS and nitric oxide levels had an inverse correlation (r= -0.59) (P< < 0.001). These relationships are illustrated in Figure 6, which demonstrates the strong association between endotoxemia and endothelial dysfunction.

Table (7): Correlation coefficients between serum LPS and selected biomarkers

Variable	Correlation Coefficient (r)	P-value
VCAM-1	0.62	<0.001
ICAM-1	0.58	<0.001
TNF-α	0.66	<0.001
IL-6	0.61	<0.001
MDA	0.55	<0.001
Nitric Oxide (NO)	-0.59	<0.001

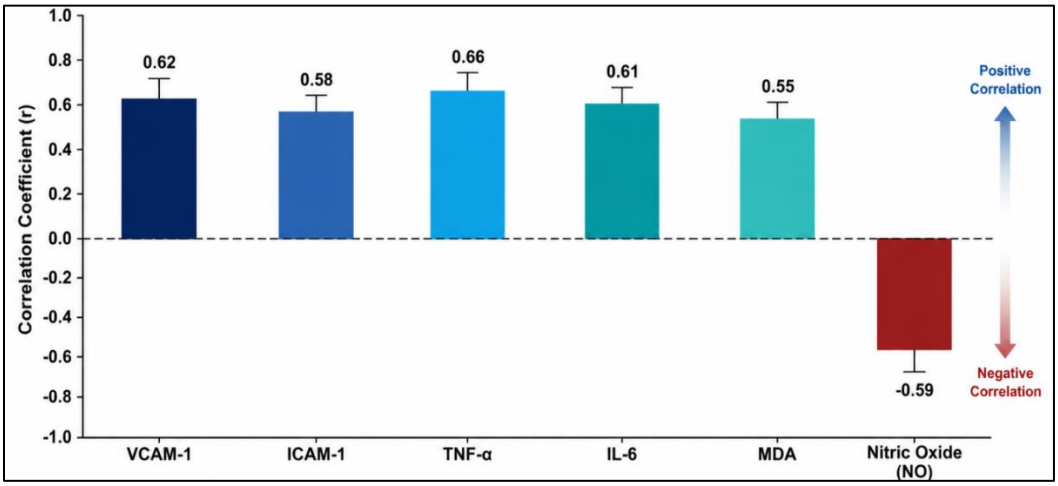


Fig (6): Correlation between serum LPS concentration and endothelial dysfunction biomarkers

3.8 Multiple Regression Analysis

Multiple linear regression analysis was carried out to establish independent predictors of endothelial dysfunction. From Table 8, it can be noted that serum LPS and TNF-α emerged as significant independent predictors of VCAM-1 levels even after adjustments were made for age, BMI, lipid profile, and glycemic parameters ($P < 0.001$). The regression analysis explained a considerable amount of variation in endothelial dysfunction biomarkers, thus reinforcing our hypothesis regarding endotoxemia and systemic inflammation playing a major role in endothelial activation.

Table (8): Multiple regression analysis for predictors of endothelial dysfunction

Variable	β-Coefficient	Standard Error	t-value	P-value
Serum LPS	0.48	0.07	6.84	<0.001
TNF-α	0.37	0.08	4.91	<0.001
Age	0.06	0.05	1.21	0.228
BMI	0.09	0.04	1.67	0.097
LDL-C	0.11	0.05	1.89	0.061

The current study examined the association between the presence of *Escherichia coli* colonization in the intestine and endothelial dysfunction markers in apparently healthy subjects [15]. It was found that those people who were colonized by *E. coli* had elevated concentrations of lipopolysaccharides (LPS), proinflammatory cytokines, oxidative stress and endothelial dysfunction markers compared to the uncolonized ones [16]. These results indicate that the colonizing *Escherichia coli* in the intestine is related to the vascular changes in apparently healthy subjects. But since this is a cross-sectional study, causality cannot be established [17]. Although the results of the current study corroborate those from some other studies, other researchers have found a negative relationship between the presence of *Escherichia coli* in the gut and endothelial dysfunction [18]. This discrepancy might be due to differences in study design, microbiological testing, and other variables [19].

One of the most significant results of the current research was the increased serum LPS level in the colonized group [20]. LPS is one of the main components of the outer cell membrane of gram-negative bacteria and serves as a strong inflammatory stimulus [21]. High serum LPS levels suggest that there is increased translocation of bacterial products into blood vessels, and this phenomenon is known as metabolic endotoxemia [22]. Some previous studies have revealed that long-term exposure to low doses of endotoxins could lead to activation of immune responses, inflammation of blood vessels, and development of cardiovascular and metabolic disorders [23]. Thus, elevated serum LPS may represent a potential biological mechanism underlying the observed association between intestinal *Escherichia coli* colonization and endothelial dysfunction [24]. One possibility in biology could be that LPS, which is a molecule released by gram-negative bacteria, stimulates TLR4 receptors that activate the NF-kB signaling pathway, causing the production of more pro-inflammatory cytokines. The inflammatory response results in increased production of adhesion molecules like VCAM-1 and ICAM-1, which leads to endothelial dysfunction [25]. In addition to this, there was a marked increase in the levels of serum VCAM-1, ICAM-1, and Endothelin-1 along with the decreased availability of nitric oxide in the colonized participants [26]. These biomarkers are known to be reliable biomarkers of endothelial activation and vascular damage [27]. Both VCAM-1 and ICAM-1 mediate the process of leukocyte adhesion and migration across the vascular wall, whereas Endothelin-1 is a strong vasoconstrictor that causes vasoconstriction leading to the dysfunction of vascular relaxation [28]. On the other hand, nitric oxide is important for maintaining vascular homeostasis because of its vasodilating, anti-inflammatory, and anticoagulatory properties [29]. Hence, the reduction in nitric oxide levels is indicative of endothelial dysfunction and loss of protective mechanisms of the vasculature [30, 31].

One of the key observations in this study is the significant rise of pro-inflammatory biomarkers, namely hs-CRP, TNF-α, and IL-6 in colonized individuals [32]. Cytokines are the principal players in initiating and driving inflammatory response [33]. It is well-established that TNF-α and IL-6 cause stimulation of the endothelium, upregulation of adhesive molecules and oxidative stress, leading to endothelial dysfunction [34]. Increased concentrations of hs-CRP confirm the existence of low-grade inflammation in individuals colonized by bacteria [35]. High level of positive correlation between LPS and pro-inflammatory biomarkers strengthens the assumption about the significant role of immune response in endotoxin-induced inflammation in colonized individuals [36].

It seems like oxidative stress may also serve as an additional pathway through which the colonization of the intestine by *E. coli* may affect vascular health [37]. It has been found that MDA concentrations in colonized individuals were markedly increased in parallel with decreased total antioxidant capacity [38]. The concentration of MDA is considered a classic indicator of lipid peroxidation, while TAC demonstrates the general antioxidant potential of biological organisms [39]. Increased oxidative stress may affect endothelial function due to reduction in nitric oxide bioavailability and inflammation [40]. Such observations agree with the results obtained previously on the role of oxidative stress in endothelial damage [41]. In addition to inflammation and oxidative stress, this current study revealed that there were major metabolic disturbances in colonized individuals [42]. Increased levels of total cholesterol, triglyceride, low density lipoprotein cholesterol, fasting glucose, fasting insulin, and HOMA-IR were detected whereas HDL cholesterol levels were significantly decreased [43]. The results imply that there could be a possible link between the colonization of bacteria such as *E. coli* and poor metabolic stability [44]. Previous research has shown that chronic exposure to endotoxins could be responsible for developing insulin resistance, dyslipidemia, and metabolic syndrome via triggering inflammatory pathway [45]. Thus, the metabolic disturbances noted in the current investigation can be considered to be related to high endotoxin levels and systemic inflammation of subjects infected with *Escherichia coli* [46]. Clinically speaking, the results of the current study indicate the association between the intestinal colonization by *Escherichia coli* and the presence of early vascular changes in seemingly healthy adults. Thus, the determination of inflammatory and endothelial biomarkers may help identify patients at risk of developing cardiometabolic complications. Nevertheless, further investigations are needed [47].

3.9 Study Limitations

There are various limitations for this study. The first one is the fact that the cross-sectional study design makes it impossible to determine the causal relations between intestinal *Escherichia coli* colonization and the development of endothelial dysfunction. The second limitation lies in the fact that patient stratification was done using traditional stool cultures; thus, low-grade colonization that cannot be found using cultures is impossible to exclude. The third limitation of the study is its single-center character, making the results less generalizable. However, the relative large sample size and the analysis of different biomarkers support the validity of the study findings.

3.10 Study Strengths

Even with these drawbacks, there are certain strengths of the current study. The study had a reasonably large number of healthy subjects with even distribution of groups. Moreover, the study involved testing for various markers of endothelial dysfunction, inflammation, oxidative stress, metabolism, and endotoxemia in order to obtain an integrated measure of a possible relationship between *E. coli* colonization and vascular health.

4- CONCLUSION

From the cross-sectional study showed a significant relationship between the presence of *Escherichia coli* in the intestines of healthy individuals and indicators of endothelial dysfunction, systemic inflammation, oxidative stress, endotoxemia, and metabolic disorders. Nevertheless, in view of the cross-sectional nature of the study, such conclusions can only be considered associations rather than causes. Further research is necessary to establish the causality between the two factors. Future research must adopt prospective longitudinal as well as multicenter study designs to establish the cause-effect relationship of intestinal *Escherichia coli* colonization and endothelial dysfunction. Moreover, application of molecular identification methods in conjunction with gut microbial community characterization and serial biomarker measurements will allow for better elucidation of the mechanisms underlying this relationship.

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