Intercellular adhesion molecules -1 (ICAM-1) and Vascular cell adhesion molecules-1 (VCAM-1) in hypertensive patients suffering from bacterial UTIs .

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Abstract

Leukocyte binding to cellular adhesion molecules (CAMs) on the surface of vascular endothelial cells appears to be one of the earliest events in the atherosclerotic process. In this study the concentration of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) on lymphocytes were evaluated during vascular disease like hypertension . In addition level of CAMs in hypertensive patients in response to other factors like sex, aging , smoking , anti hypertension drugs treatment (mainly with some β-blockers(propranolol) or ACE inhibitors(captopril)) and UTIs were also measured . As compared to controls mean of ICAM-1 and VCAM-1 were significantly elevated in hypertensive subjects. VCAM-1 was significant in male compared to female . Significant positive correlation was observed of VCAM-1 level in relation to ageing , Smoking and in hypertensive patients suffering from UTIs. While the level of both ICAM-1 and VCAM-1 was found to be decreased upon anti hypertension treatment with either β-blocker or ACE inhibitor decreased.

جزيئات الالتصاق الخلوية (VCAM-1 and ICAM-1) عند مرضى أرتفاع ضغط الدم اللذين بعانون من التهاب المسالك البولية .

الخلاصه :

أشكال جزيئات الالتصاق الخلويه (CAMs) قد تكون من العلامات المفيدة الناتجة عن تنشيط الخلايا البطانية أو تنشيط الالتهابات التي قد تكون شامله أو ضمن أطار محدد . في هذه الدراسة وجد أن تركيز جزيئه الالتصاق الخلوية (ICAM-1) وجزيئه التصاق الخلوية الوعائية (VCAM-1) المرتبطة في الخلايا اللمفاوية مع بعض الأمراض الوعائية مثل ارتفاع ضغط الدم قد ارتفعت بشكل معنوي . كما أظهرت النتائج زيادة جزيئات الالتصاق الخلوية الوعائية (VCAM -1) في الرجال مقارنة بالنساء وفي حالات الشيخوخة ,التدخين في نموذج السيطرة الموجبة وعند المرضى أللذين يعانون من التهاب المسالك البولية . في حين انخفضت بشكل معنوي في حالة العلاج بأحد أدوية علاج الضغط مثل (β-blocker or ACE inhibitor) .

المفاتيح : جزيئات الالتصاق الخلويه , ارتفاع ضغط الدم , الشيخوخة , التدخين , التهاب المسالك البولية .

Introduction

Hypertension or high blood pressure is a cardiac chronic medical condition in which the systemic arterial blood pressure is elevated. Hypertension is classified as either primary (essential) hypertension or secondary hypertension; About 90–95% of cases are categorized as "primary hypertension," which means high blood pressure with no obvious medical cause (1) . The remaining 5–10% of cases (Secondary hypertension) are caused by other conditions that affect the kidneys, arteries, heart or endocrine system (2) . In addition to the kidney, the vasculature, and the central nervous system, accumulating evidence indicates that the immune system contributes to hypertension. Cell Adhesion Molecules (CAMs) are proteins located on the cell surface involved with the binding with other cells or with the extracellular matrix (ECM) in the process called cell adhesion. These proteins are typically transmembrane receptors and are composed of three domains: an intracellular domain that interacts with the cytoskeleton, a transmembrane domain, and an extracellular domain that interacts either with other CAMs of the same kind (homophilic binding) or with other CAMs or the extracellular matrix (heterophilic binding). Leukocyte binding to cellular adhesion molecules (CAMs) on the surface of vascular endothelial cells appears to be one of the earliest events in the atherosclerotic process (3). Increased endothelial cell expression of CAMs has been demonstrated in response to a number of inflammatory cytokines, including interleukin-1, interleukin-4, tumor necrosis factor-alpha, interferon-gamma, lipopolysaccharide and oxidized low density lipoprotein (LDL) (4). Pathologic studies have demonstrated CAMs within and adjacent to atherosclerotic plaque , and clinical studies have suggested a role for CAMs in plaque disruption and subsequent acute coronary events (5). After cytokine activation, CAMs are released from the surface of endothelial cells and leukocytes, probably by proteolytic cleavage . Although the pathogenic role of these CAMs in disease states remains unclear, these molecules may serve as markers of endothelial activation and local or systemic inflammation . For example, in cross-sectional studies, the plasma concentrations of intercellular adhesion molecule-1(ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) correlate with the extent of underlying atherosclerosis (6) . Intercellular adhesion molecule-1 (ICAM-1) is a likely product of many cells, including the endothelium and leukocytes. Also influenced by inflammatory cytokines in vitro , raised levels are found in many conditions, including angina and both coronary artery disease and peripheral artery disease (51). However, although raised levels in healthy men predict adverse events . Vascular cell adhesion molecule-1 (VCAM-1) seem to be increased in the plasma of patients with angina or coronary artery disease (7).

Patients and Methods :

Sixty five patients examined in the cardiovascular and urology unit (40 male and 25 female), with a mean age of 50 years (range 30-70 years) were chosen ,they were all suffering from clinical manifestation of hypertension . The diagnosis in each case was established by clinical examination done by specialist doctor in AL-Kadhimyia teaching hospital in Baghdad hospital from March to December 2011.
Twenty-five patients (15 male and 10 female) were presented with only hypertension disease (hypertension for the first time) .While the rest Twenty patients (15 male and 5 female) were presented with hypertension (which mainly treated by either ACE inhibitor or simple beta blocker). Finally, twenty patients (15 male and 5 female)were presented with hypertension and UTIs. Thirty apparently healthy volunteers (15male and 15 female) with the mean age 42 years and age range (24-60) years were enrolled as control.

Sample collection:

Blood samples were obtained after an overnight fast for measurement of ICAM-1 and VCAM-1 . From each patient and control ,five ml venous blood was aspirated from a suitable vein efficient disinfecting over the injection site. blood samples were immediately transferred to sterile heparinised vacutainer tubes for lymphocyte separation .

Lymphocyte separation :

The Isopaque-ficol technique originally described by Boyum (8) was used for lymphocyte separation .

Direct immune flourescence staining technique for the detection of ICAM-1 and VCAM-1 :

Flourescen isothiocynate (FTIC) labeled monoclonal antibodies directly react with antigens on the surface of separated lymphocytes . FTIC is molecules that have the ability to absorb invisible, ultraviolet radiation, and then have the capability of emiting an apple green fluorescence colored light after exposure to UV light (9) .

Direct immunoflourescence procedure :

The method of IF-labeling of fixed cells was done as described by Batty (6) as following :

The procedure should be done in safety cabinet hood for complete sterile conditions .

1-Pre-collected of heparinized blood was diluted in equal volume of PBS (2ml from heparinized blood +2ml from PBS), this mixture then quietly over layered on 4 ml of Isopaque –Ficoll (Lymphocyte separation medium) by using Pasteur pipette, centrifuged for 15 min at 3000 rpm.

2-After centrifugation, a white turbid layer of lymphocytes accumulated on top of the isopaque-ficoll layer, erythrocytes sediment to the bottom of the tube. Then the layer of lymphocytes was aspirated into another sterile tube by using Pasteur pipette and centrifuged for 10 min at 3000 rpm and the supernatant was discarded as before .

3-The cell pellet was resuspended by using 0.5 ml of PBS .

Red blood cells (RBCs) lysis: RBCs lysis technique was found necessary in many patients and control blood samples during lymphocytes isolation step, because those RBCs are the major contaminant elements with the isolation lymphocytes in density gradient technique.

Cell counting and viability test (10)

The final peripheral blood lymphocytes (PBLs) suspension was diluted 1:10 with 0.5% trypan blue dye then mixed well by Pasteur pipette. A sufficient amount of final PBLs suspension was aspirated to fill the chamber by the capillary phenomenon that exerted by the minute space between chamber floor and overlying cover slip. By using light microscope, we can count the number of viable lymphocytes, which were unstained ones and ignore the stained ones, because they were the dead cells. Counting the mean of viable cell number in the large squares of chamber. Applying the following formula to obtain the final lymphocytes concentration :

The final lymphocytes concentration /ml=M$\times10^4\times$ dilution factor

M=mean of cells number per large square ;

10^4=factor of size difference

Dilution factor = 10

- **Determination of viability**

Viability =(number of viable cells/number of viable cells+ number of dead cells)$\times$100

The accepted viability should be 95% and above .After the calculation of viability and concentration of isolated lymphocytes, these cells were fixed on IF-slides.

Fixation step : The fixation procedure dissolves and removes the lipids, so that the cellular proteins, both surface and intracellular and nucleic acids are accessible to added antibodies. While fixation is critical for cell adhesion to immunfluorscence slides and to be sticky apart from the frequent washing steps later on (11).

— **Urine collection** : Proper collection of urine specimen is the most important step in urine culture , and prevention of contamination is the most important consideration for collection of urine sample (12). Patients were instructed to wash their outer genitalia with water which was consider satisfactory and preferable than other antiseptic in the form of soap or other chemicals .Mid stream urine (MSU)samples were collected in clean and sterile screw capped containers .

— **Cultivation and microscopic examination of urine specimens :**The urine specimens were cultured immediately (12) . The urine specimens were inoculated on both blood and MacConkey agar plates by direct streaking method using calibrated loop to deliver 0.01 ml of the urine specimen (diameter of the loop is 4mm) . The plates were incubated at 37C for 18-24 hours then examined for bacterial growth . If there was no growth ,the plates were reincubated for another 24 hours before they were discarded as negative culture (13).

Statistical Analysis
Statistical analysis were conducted to describe different variables and parameters in this research and to describe relationships with each other as well. Calculation of means ± standard errors was done for quantitative data. Sensitivity and specificity were also calculated to specify and compare the efficiency of different diagnostic tests which was done by using SAS computer analysis program (14).

Results
Table 1 showed the distribution of cell adhesion molecules (ICAM-1 and VCAM-1) among hypertensive patients (Fig 1) and normal individual . Intercellular adhesion molecules (ICAM-1) values showed significant difference ($p \leq 0.01$) with mean value of (52.68±0.91) compared to normal control (45.2 ± 0.41) , for VCAM-1 , a highly significant difference ($p \leq 0.01$) and mean value of (65.63 ± 0.67) for hypertensive patients compared to normal control (58.4 ± 1.2) .

Table 1 : **Cell adhesion molecules ICAM-1 and VCAM-1 in hypertensive patients and normal control .**

Patients	ICAM-1	VCAM-1
Hypertension	52.68 ± 0.91 A	65.63 ± 0.67 A
Normal control	45.2 ± 0.41 B	58.4 ± 1.2 B
Significance	$P \leq 0.01$	$P \leq 0.01$

Note : Means with the same letter are not significantly different .

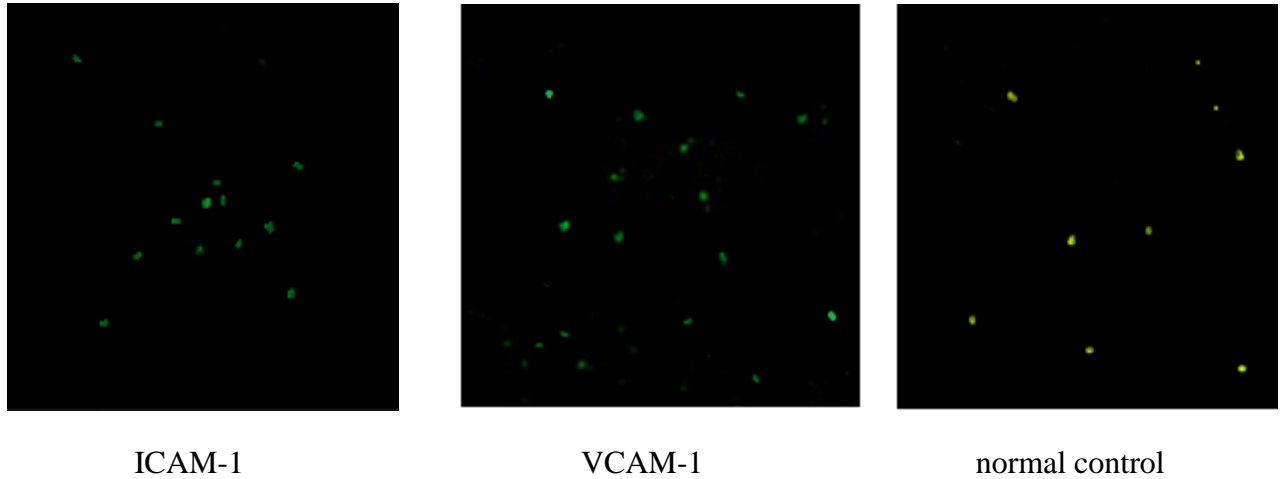| ICAM-1 | VCAM-1 | normal control |

 Figure-1: Immunoflourescent VCAM-1 and ICAM-1 compared to normal control (hypertensive patients).

Cell adhesion molecules and gender :

 Table 2 show the frequency distribution of serum ICAM-1 among male and female which show no significant difference ($p \geq 0.8$) and mean values was relatively equal both in male and female , while serum VCAM-1 show a significant differences ($p \leq 0.01$) with mean value of (67.57 ± 0.89) in male and (62.72 ± 0.81)for female .

Table 1-2   Effect of gender on level of ICAM-1 and VCAM-1 in hypertensive patients .

SEX	ICAM-1	VCAM-1
Male	52.42±1.19 A	67.57±0.89 A
Female	52.95±1.42 A	62.72±0.81 B
Significance	P≥ 0.8	P≤ 0.01

Note : Means with the same letter are not significantly different.

Cell adhesion molecules and aging :

Table -3 show the distributions of cell adhesion molecules among different age groups of hypertensive patients , ICAM-1 expression was found to be not affected (no significant differences) during hypertension disease (p ≥ 0.1) , while VCAM-1 show a high significant differences   (p ≤ 0.01) and the rate of expression start to increase gradually during age group of (30 - 40) years and reach highest level during old age group (≥61 years) .

Table 3 : Distribution of cell adhesion molecules ICAM-1 and VCAM-1 in hypertensive patients with different age groups .

Age /year	ICAM-1	Control	VCAM-1	Control
30 – 40 y	45.95 ± 2.29 B	44.21±1.98	61.74 ± 1.48 C	56.22±2.45
41 – 50 y	53.09 ± 1.53 A	53.11±0.04	64.25 ± 0.99 B	60.03±1.01
51 – 60 y	53.57 ± 1.44 A	52.23±2.01	64.02 ± 1.06 B	59.01±1.99
≥ 61 y	53.62 ± 2.12 A	52.75±3.06	70.73 ± 1.42 A	65.72±0.54
Significance	P ≥ 0.1	P ≥ 0.05	P ≤ 0.01	P ≤ 0.01

Note : Means with the same letter are not significantly different .

Cell adhesion molecules and smoking :

Smoking versus expression of cell adhesion molecules (ICAM-1 and VCAM-1) in hypertensive patients was measured and the results showed a significant differences (p≤ 0.01) both in VCAM-1 and ICAM-1   expression with mean value of (68.21±1.09) and (58.38 ± 1.45) for smoking patients compared to (65.63±0.67) and (52.68 ± 0.91) for control respectively (Table-4).

Table -4 : Effect of smoking on expression of cell adhesion molecules ICAM-1 and VCAM-1 in hypertensive patients .

Hypertensive Patients	ICAM-1	VCAM-1
Control	52.68 ± 0.91 B	65.63 ± 0.67 B

Smoking	58.38 ± 1.45 A	68.21±1.09 A
Significance	P ≤ 0.01	P ≤ 0.01

Note : Means with the same letter are not significantly different.

Role of some anti hypertensive drugs (propranolol and captopril) on expression of ICAM-1 and VCAM-1 :

The other group which was measured is the role of some anti hypertensive drugs (mainly beta blocker or angiotensin converting enzyme inhibitor) on expression of serum cell adhesion molecules (ICAM-1 and VCAM-1) (Table-5). The table show for both types of anti hypertensive drugs a significant decrease on expression of cell adhesion molecules was observed [for ICAM-1and VCAM-1 value ($p \le 0.05$)($p \le 0.01$), with mean value of (46.5±1.52) and (53.05±0.92) for beta blocker and (48.68±1.1) and (58.5±1.08) for angiotensin converting enzyme inhibitor compared to mean value of other hypertensive patients whom welling to take anti hypertension medication for the first time (52.68±0.91) and (65.63±0.67) respectively.

Table -5   Role of antihypertensive drugs (mainly beta blocker and Angiotensin converting enzyme inhibitor) on expression of cell adhesion molecules (ICAM-1, VCAM-1) .

Treatment	ICAM-1	VCAM-1
B.blocker *	46.5±1.52 C	53.05±0.92 C
ACE inh. *	48.68±1.1 B	58.50± 1.08 B
Positive control	52.68±0.91 A	65.63±0.67 A
Negative control	45.2 ± 0.41 C	58.4 ± 1.2 B
Significance	P≤ 0.05	P≤ 0.01

B.blocker : Beta blocker ; ACE inh. :Angiotensin converting enzyme inhibitor.

Note : Means with the same letter are not significantly different.

Urinary tract infections and cell adhesion molecules expression :

Table-6 showed a significant differences in ICAM-1 and VCAM-1 level ($p \leq 0.01$) with mean value of (58.87 ± 1.22) and (68.24 ± 1.16) for hypertensive patients having bacterial UTI compare to mean value of (52.68 ± 0.91) and (65.63 ± 0.67) for positive control and (45.2 ± 0.41) and (58.4 ± 1.2) for negative control respectively .

Table -6 . Effect of bacterial UTI on expression of ICAM-1, VCAM-1 in hypertensive patients.

Hypertensive Patients	ICAM-1	VCAM-1
With UTI	58 .87± 1.22 A	68.24±1.16 A
Positive control	52.68±0.91 B	65.63±0.67 B
Negative control	45.2 ± 0.41 C	58.4 ± 1.2 C
Significance	P ≤ 0.01	P ≤ 0.01

DISCUSSION :

Hypertension is a well-known risk factor for cardiovascular disease, but the pathologic and molecular mechanisms by which elevated blood pressure leads to vascular disease are uncertain. There is some experimental evidence, however, to suggest that hypertension may promote endothelial expression of cytokines and stimulate inflammation. These data are particularly intriguing given the growing evidence that inflammation plays a critical role in the pathogenesis of atherosclerosis (15) . The endothelium is a favourite early target of cardiovascular risk factors and cardiovascular diseases like hypertension. This key role of the endothelium in terms of cardiovascular risk results from its capacity to respond to numerous autocrine and paracrine stimuli, because of the presence on these cells of numerous membrane receptors (eg muscarinic, serotoninergic, adrenergic, cytokines, growth factors, etc) or intracellular receptors (eg oestrogens) (16). Cell adhesion molecules play an important role in this initial phase . The blockade of the interaction between leukocytes and the endothelium by agents that mimic or inhibit these adhesion molecules may become a new class of therapeutic agents (17), study of the expression of the molecules on the surface of various cells or of the soluble form in the plasma may provide insights into their role(s) in pathophysiology and measurement levels of cell adhesion molecules may be useful tools in stratifying disease severity or prognosis . Inflammation, where by adhesion molecules play an important role, has been implicated in the pathogenesis of hypertension (18).

Cell adhesion molecules (ICAM-1, VCAM-1) and hypertension :

Regarding ICAM-1 level the result of the present study came in agreement with (19), (20) they proposed that damage to the endothelium is important in the development and/or progression of atherosclerosis and hypertension disease , and increased levels of endothelial cell markers in the plasma of subjects who go onto suffer adverse cardiovascular events . It was also noted that significantly higher levels of ICAM-1, von Willebrand factor markers (vWf) , and endothelin-1 among the hypertensives compared to the normotensives patients these Evidence suggests that numerous haemorheological, platelet and endothelial factors may contribute to the pathogenesis of hypertension.

Stéphanie (3) reported that high pressure increases the endothelial expression of ICAM-1 and induces a strong over expression of VCAM-1, MCP-1 and IL-6 in all vascular cells, through induction of NF-κB. He also demonstrated that monocyte adhesion occurs via the interaction of $\alpha_4\beta_1$ integrins with VCAM-1 rather than ICAM-1. However, blockade of MCP-1, IL-6 led to a strong decrease in monocyte adhesion to the vascular wall, and in vitro studies confirmed that all these cytokines are necessary to prime monocyte adhesion.

Lee (21) authorized that endothelial dysfunction has also been associated with increased expression of cell adhesion molecules (E-selectin, Pselectin, ICAM-1 and VCAM-1.Our study was agreed with (24) studies which suggested that hypertension enhances responsiveness of endothelial cells to factors that promote monocyte/macrophage adhesion, because cytokine- and endotoxin-stimulate ICAM-1 expression and macrophage infiltration are more intense on endothelial cells derived from spontaneously hypertensive rats compared with cells from normotensive .

There is increasing evidence that inflammatory responses lead to alterations of the chemotactic and adhesive property of endothelial cells that support monocyte/macrophage chemotaxis, adhesion, and transmigration into the vessel wall. Recent studies suggested that pressure overload itself is a strong proinflammatory factor because mechanical strain can induce inflammatory cytokines, growth factors, and oxidative stress in the resident cells of the vessel wall, including endothelial cells, smooth muscle cells and inflammatory cell infiltrates. The interplay of these factors may regulate ICAM-1 expression in aortic constriction rats (21) . The level of another cell adhesion molecules (VCAM-1) was evaluated in this study which was correspond with (22) study who reported that increased blood pressure was apart from various pathological changes that involve increases in production of free radicals by angiotensin II . Incomplete oxidation and enhanced production of free radicals may be associated with medial thickening, which could prolong the diffusion of oxygen from the vascular lumen (5).This increased production of free radicals in endothelium may trigger a cascade activating the transcription factor nuclear factor kappa B (NFkB), which in turn regulates expression of molecules such as ICAM-1 and VCAM-1. Thus expression of CAMs may be regulated at the transcription level that may consequently influence concentration of CAMs (23) . The increases in level of ICAM-1 and VCAM-1 in hypertensive patients as shown in our study is also documented by (24) whom mentioned that It is commonly accepted that long term impact of proatherogenic factors on vascular endothelium results in chronic, subclinical inflammation with a consequent rise in vascular and plasma concentrations of several acute-phase reactans such as IL-6 . Persons with hypertension have higher circulating levels of ICAM-1,VCAM-1 , IL-6, TNF-α, and fibrinogen than non-hypertensive individuals, supporting a possible role of hypertension as a proinflammatory stimulus . It has been suggested that a rise in blood pressure activates a vicious cycle, causing chronic inflammation of the endothelium, which in turn might be responsible for a further damage of endothelium and worsening of blood pressure control (25),(26) .

Gender and age what do they reflect on CAM expression :

A profile of soluble adhesion molecule level may allow better therapeutic decisions in inflammatory and autoimmune disorders, infection, cancer, and cardiovascular pathologies and may also aid in the prediction of cardiovascular events . However, the use of these markers in clinical practice depends critically on knowledge of their reference values. In this study differences between

male and female were investigated and the results showed that the frequency distribution of ICAM-1 among male and female show no significant difference ($p \geq 0.8$), while VCAM-1 level showed a significant differences ($p \leq 0.01$). Such results were in line with report done by Huo (28) whom mentioned that level of ICAM-1 and P-selectin concentrations in hypertension patients did not vary significantly across all age groups, independent of gender, and are probably partly attributable to steroid hormones, especially estrogen. Another study by Elhadd (29) investigating levels of CAMs in three different phases of the menstrual cycle in young healthy normal cycling women reported that changes in estrogen levels during the menstrual cycle can significantly alter the levels of E-selectin, but not ICAM-1.

Bonello (30) also mentioned that Female sex hormones are known to exert a protective role on the vascular endothelial function, but the exact mechanisms of such protection are not known. Studies of the possible regulatory role of female sex hormones changes during the normal menstrual cycle on soluble adhesion molecules are rare and contradictory. The levels of ICAM-1 did not vary through the cycle, but the mean percentage change in E selectin was significant between early follicular and luteal phases. One of the goals of the present study is to analyze the correlation of CAM with aging, Aging is associated with several structural and functional modifications of the vascular endothelial wall. These changes occurring with aging may contribute to an increased susceptibility to develop hypertension lesions. However, the mechanisms by which aging acts are still unknown. The predisposition of the old vessels to develop hypertension lesions may be related to an age dependent increase of the expression of cell adhesion molecules (CAMs) on the surface of vascular endothelial cells. CAMs, such as VCAM-1, ICAM-1, and endothelial leukocyte adhesion molecule-1 (E-selectin), are known to modulate cell-endothelium interactions The expression of these CAMs is suspected of playing in a key role in adhesion and trans endothelial migration of monocytes which results in the formation of fatty streaks(2). Upregulation of CAMs is accompanied by the release of forms of adhesion molecules into the bloodstream. Therefore, increased levels of circulating CAMs (cCAMs) have been suggested as indexes of elevated CAM expression. The levels of cCAMs may be useful markers for stratifying cardiovascular disease severity or prognosis (31).Our result was also agreed with (54)Study which showed the concentration of ICAM-1 and VCAM-1 to be significantly greater in normal children than in adults, and these concentrations were found to decline with age while the reverse is true in inflammatory disease like CHD, hypertension and atherosclerosis .

In the long-term study of (17) they found a significant rise in the adhesion molecule VCAM-1 in apparently healthy males who had a heart attack during a 9-year period of follow-up. This unique observation would appear to confirm the hypothesis that endothelial cell dysfunction and chronic inflammation of the vessel wall develop early, many years preceding the onset symptoms of coronary heart disease. Although other studies did not agree with our result including of (32),(33) which suggested that levels of E-selectin, ICAM-1, and VCAM-1 are constant with age between 18 and 65 years, although few details are given .Michele (34) findings suggested that the increase in concentrations of some adhesion molecules like (VCAM-1) in old age hypertensive patients may be a sign of simple endothelial activation secondary to stress to vessels caused by blood pressure increase when compare to young patients .

Smoking and Cell adhesion molecules :

The result of this study was agreed with Shahar (35) who measured other systemic inflammatory biomarkers and found that smokers had higher levels of lipoprotein-associated phospholipase A2 (Lp-PLA2), myeloperoxidase, and ICAM-1 than nonsmokers and a similar levels of C-reactive protein, VCAM-1, and fibrinogen. Thus, it appears that smoking may have a differential effect on inflammatory pathways that play a role in endothelial dysfunction . Other study by Mazzone (22) also reported a significant increase in the levels of VCAM-1 in smoking patients with hypertensive disease and suggested smoking as a cause for endothelial dysfunction . Some studies certainly suggested that elevated ICAM-1 is a suitable marker of vascular disease status and a risk factor for future acute vascular events (7), (17), and that these significant associations are not confounded by

smoking habits. Nevertheless, it has been clearly established that a dramatic, dose-dependent increase in circulating ICAM-1 levels is one consequence of tobacco smoking. Therefore smokers will be more likely to be in the upper quartile of ICAM-1 levels, where increased risk of vascular disease is most evident. Indeed, the influence of smoking on ICAM-1 concentration is commonly reported to be as great, or greater as that ascribed to disease .Among all smokers circulating levels of ICAM-1 and VCAM-1 were significantly elevated, compared to their non-smoker counterparts . As for the impact of hypertension, there was a significant increase in the levels of both ICAM-1 and VCAM-1 in association with hypertension, compared to their normotensive . Moreover, the level of increase in ICAM-1 and VCAM-1 that was observed in association with hypertension alone was less than that observed in association with cigarette smoking alone, which might reflect a difference in the mechanism of elevation. Furthermore, the association of both hypertension and cigarette smoking significantly boosted the levels of ICAM-1 and VCAM-1 above that observed with either one alone (36). Blann (37) reported that, compared to appropriate control groups, VCAM-1 is significantly elevated in the smokers with peripheral artery disease, but not healthy smokers, implying an indirect relationship. These observations are in line with the results of this study showing that association of both hypertension and cigarette smoking significantly boosts the levels of markers of endothelial dysfunction (mostly ICAM-1 and VCAM-1) level . Elsewhere, (9) study was not agreed with our study he found that VCAM-1 levels were actually lower in male smokers, compared to non-smokers, but this was not true for females, such results may be related to small sample size .

Relation of anti-hypertension(mainly ACEI or Beta blocker) medication and expression of cell adhesion molecules :

Treatment with anti hypertensive treatment was included in this study and the results showed a significant effect on expression of cell adhesion molecules (ICAM-1 and VCAM-1) level .Such results were in line with (38) who reported that renin-angiotensin system (RAS) blockade by ACEI has a number of potentially anti atherogenic effects, eg, reduction of expression of adhesion molecules and chemokines, subintimal macrophage infiltration and macrophage-mediated oxidation of LDL, deactivating of NF-κB, upregulation of peroxisome proliferator-activated receptors, and increase of vascular nitric oxide release. Indeed, ACEI reduced the aortic expression of VCAM-1 mRNA and the plasma concentrations of VCAM-1 , suggesting that reduced inflammatiaon in the arterial wall contributes to the anti-atherogenic effect of RAS blockade. Our result was also in line with (4) who point out that for similar degrees of hypertension, Ang II induced much greater vascular VCAM-1 gene expression than norepinephrine. These results suggest that although hypertension alone could play some role in upregulating VCAM-1 expression, it is not sufficient to fully activate vascular VCAM-1 expression. These studies suggested that Ang II and not hypertension alone may initiate oxidative signaling mechanisms; these mechanisms in turn activate redox-sensitive transcription factors such as NF-κB and upregulate the expression of NF-κB driven genes such as VCAM-1. They demonstrated that Ang II induces VCAM-1 gene expression at physiological concentrations, which are similar to those in other reports of Ang II actions. In endothelial and vascular smooth muscle cells, cytokine mediated VCAM-1 gene expression occurs through NF-κB mediated transcriptional mechanisms (39). Eero (40) found a significant monocyte/macrophage infiltration in the renal perivascular space and increased expression of in ICAM-1 and VCAM-1 in the interstitium, intima, and adventitia of the hypertensive small renal vessels. Their findings indicate that angiotensin II causes monocyte recruitment and vascular inflammatory response in the kidney by blood pressure–dependent and blood pressure–independent mechanisms. ACE inhibition, AT$_1$ receptor blockade, and human renin inhibition all prevent monocyte/macrophage infiltration and decreased adhesion molecule expression in the kidneys (41) .

Cell adhesion molecules expression and urinary tract infection :

Persistent microbial infections are a rapidly expanding problem because of increased antimicrobial resistance . This trend is particularly concerning because of the increasingly appreciated role that chronic infections may play in cancer and chronic inflammatory diseases . One key example is that of urinary tract infections (UTI), which are common, highly recurrent, and can become chronic .

In the present study the result shows a significant differences in ICAM-1 and VCAM-1 levels for hypertensive patients having bacterial UTI compared to hypertensive patients without UTI such result was in line with Douglas(42) who mentioned that Gram-negative bacterial sepsis remains a common, life-threatening event , including the development of acute urinary tract infections. A common finding among patients and experimental animals with UTI is endothelial injury and/or dysfunction . A component of the outer membrane of gram-negative bacteria, lipopolysaccharide (LPS) or endotoxin, has been implicated in the pathogenesis of much of the endothelial cell injury and/or dysfunction associated with these disease states. LPS is a highly proinflammatory molecule that elicits a wide array of endothelial responses, including the upregulation of cytokines, adhesion molecules (ICAM-1,VCAM-1) and tissue factor (43). In addition to activation, LPS induces endothelial cell death that is apoptotic in nature. However Endothelial apoptosis has been implicated in the pathogenesis of several disease states, including atherosclerosis, hypertension , congestive heart failure , and systemic capillary leak syndrome. Several studies have reported that endothelial cell injury and/or death is a key pathological finding during bacterial sepsis (44) . Our results was also agreed with (1) study. He authorized that   Leukocyte adhesion molecules are important for migration of the inflammatory cells into sites of inflammation. ICAM-1 and VCAM-1 are expressed in normal kidney and their expression is up-regulated in the renal tissue of hypertensive patients with bacterial kidney infection. The significantly high urinarylevels of the adhesion molecules in such disease with advanced histological changes may reflect their renal tissue expression and therefore the severity of the nephritis. Renal tissue damage in these cases may be the result of transmigration of activated inflammatory cells, inducing serious tissue damage (33) .

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