TUMOR NECROSIS FACTOR ALPHA AND INTERLEUKIN 6 IN ESSENTIAL HYPERTENSION

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ABSTRACT

Background: Epidemiological and experimental studies have revealed an association between biochemical markers of systemic inflammation and cardiovascular disease such as atherosclerosis, heart failure, and hypertension. Increased production of tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) which might play an important role in the initiation and progression of hypertension by a variety of mechanisms such as giving rise to contraction of vessels, leading to thickening of vascular wall, and proliferation of endothelial cells and smooth muscle cells. Aim: The aim of this study to evaluate the concentration of TNF-α and IL-6 in essential hypertension and correlate it with duration of hypertension and others study variable.

Material and methods: 250 patients with essential hypertension and 250 healthy individual as control. Serum and urine were collected after fasting 8 hours overnight; serum used for measurement of TNF-α, IL-6 (by using ELISA technique). And urine used for estimation of microalbumin/creatinine ratio (MCR) by turbidimetryspectrophotometry method. SPSS 20 software and Microsoft Office Excel were used for statistical analysis. Result: The mean value of TNF, IL-6 and MCR were significantly increased in patients than controls, with (P-value 0.000). Highly significant correlation between IL-6 and age, blood glucose, urea, creatinine, duration of hypertension and SBP. No correlation between IL-6 and gender, BMI and DBP. Also there were highly significant correlation between TNF and age, blood glucose, urea, creatinine, duration of
hypertension and SBP. No correlation between IL-6 and gender, BMI and DBP. There were significant correlation between MCR and gender, age, blood glucose, urea, creatinine, and duration of hypertension. No correlation between MCR and BMI, SBP and DBP.

**Conclusion:** TNF-alpha and IL-6 also are significantly increased in hypertension and may use in early detection of renal disease in hypertensive patients.

**INTRODUCTION**

Hypertension (HTN) also known as high blood pressure, is a long term medical condition in which the blood pressure in the arteries is persistently elevated.[1] High blood pressure usually does not cause symptoms.[2] Long term high blood pressure; however, is a major risk factor for coronary artery disease, stroke, heart failure, peripheral vascular disease, vision loss, and chronic kidney disease.[3, 4] High blood pressure is classified as either primary (essential) high blood pressure or secondary high blood pressure.[5] About 90–95% of cases are primary, defined as high blood pressure due to non specific life style and genetic factors.[5, 6]. Life style factors that increase the risk include excess salt, excess body weight, smoking, and alcohol.[2, 5] The remaining 5–10% of cases are categorized as secondary high blood pressure, defined as high blood pressure due to an identifiable cause, such as chronic kidney disease, narrowing of the kidney arteries, an endocrine disorder, or the use of birth control pills.[5]

Important blood pressure-regulatory systems, such as the renin-angiotensin system and sympathetic nervous system, interact with the pro-inflammatory cytokines, such as IL-6 and TNF-α. The sympathetic nervous system stimulates the release of pro-inflammatory cytokines, and sympathetic nerves may also serve as a source of cytokines.[7] There is also experimental evidence that pro-inflammatory cytokines may activate the sympathetic nervous system.[7] ANG II enhances the synthesis of TNF-α and IL-6 and stimulates chemokine monocyte chemoattractant protein-1 and nuclear factor-κB.[8, 9, 10, and 11] ANG II also increases the production of reactive oxygen species, including hydrogen peroxide that participates in the process of inflammation.[10, 11] Pro-inflammatory cytokines also affect vascular function and endothelium-derived factors involved in blood pressure regulation. TNF-α and IL-6 have both been shown to induce structural as well as functional alterations in endothelial cells.[12, 13 and 14] These cytokines enhance the formation of a number of endothelial cell substances, such as endothelin; reduce acetylcholine-induced vasodilatation; and destabilize the mRNA of endothelial nitric oxide synthase.[12, 13, 14 and 15] Thus endothelial dysfunction associated with many forms of hypertension may, in part, be mediated by pro-inflammatory cytokines. The
consequent activation of the intrarrenal Rennin Angiotensin System (RAS) and the increased release of cytokines and growth factors with recruitment of inflammatory cells stimulate apoptosis causing loss of normal kidney cells and increased matrix production, finally leading to progressive glomerular and interstitial fibrosis and scarring.

Increased glomerular perfusion and elevation of glomerular capillary pressure resulting in hyper-filtration, lead to further damage of the affected glomerulus\textsuperscript{[16]} and to increased filtration of proteins to the tubular lumen. Enhanced tubular reabsorption induces the synthesis of inflammatory and fibrotic factors, resulting in tubulointerstitial ischemia, inflammation, up-regulation of oxidative stress and epithelial-to-mesenchimal trans differentiation eventually culminating in fibrosis\textsuperscript{[17],[18]} and\textsuperscript{[19]}. The involvement of inflammation in the progression of CKD was widely demonstrated in experimental models of non-immunologic kidney disease\textsuperscript{[20 and 21]}

**MATERIALS AND METHODS**

This study is descriptive analytical case control study, which conducted in Khartoum state, capital of the Sudan. A number of 500 subjects were enrolled in this study, they classified into two groups, 250 patients clinically diagnosed as essential hypertension 92 (36.8%) of patients were male and 158 (63.2%) were female, and 250 healthy subjects as control 101 (40.4%) of them were male and 149 (59.6%) were female. At age from 24 years to 80 years, patients with secondary hypertension or other disease were excluded. 92 (36.8%) of patients were male and 158 (63.2%) were female. The specimens include urine and serum; Fasting urine sample for microalbumin–creatinine index, and 5 ml of venous blood was collected after 8 hours of fasting and divided into fluoride oxalate container for glucose and an plain container (without anticoagulant) for analysis of the other parameters. After allowing stand at room temperature for one hour to obtain serum (centrifuge at 3000 rpm for 10 min), then serum kept at – 30°C for TNF-α, interleukin 6 (IL-6). Blood urea, glucose and creatinine were measured immediately. The concentration of TNF-α, interleukin 6 was measured using ELISA technique. Concentration of microalbumin was measured by turbidimetry spectrophotometry method; creatinine concentration in urine was measured using kinetic Jaffe method. Then the microalbumin/creatinine ratio calculated by dividing microalbumin over creatinine. Also the concentration of blood glucose, urea, creatinine, were measured by automatic analyzer ACCENT-200. For internal quality control, normal and pathological control was been used.
Permission to carry out this research will be obtained from health authorities. Patients will be fully informed about this work.

**Statistical analysis**
For data analysis the Statistical Package for Social Science (SPSS-20) and Microsoft Office Excel will be used.

**RESULTS**
There was significant increase in the mean value of MCR, IL-6 and TNF in patients than controls, with (P-value 0.000) (fig 1).

**Fig (1):** represents the MCR, IL-6 and TNF in patients and controls.

There was a highly significant correlation between MCR and gender, age, blood glucose, urea, creatinine, and duration of hypertension. No correlation between MCR and BMI, SBP and DBP (table 1).

**Table (1): correlation between MCR and study variables.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>R-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>0.996</td>
<td>0.000</td>
</tr>
<tr>
<td>Age in years</td>
<td>0.231</td>
<td>0.000</td>
</tr>
<tr>
<td>FBG in mg/dl</td>
<td>0.176</td>
<td>0.005</td>
</tr>
<tr>
<td>Urea in mg/dl</td>
<td>0.639</td>
<td>0.000</td>
</tr>
<tr>
<td>Duration in year</td>
<td>0.336</td>
<td>0.000</td>
</tr>
<tr>
<td>BMI</td>
<td>0.009</td>
<td>0.891</td>
</tr>
<tr>
<td>SBP</td>
<td>0.120</td>
<td>0.057</td>
</tr>
<tr>
<td>DBP</td>
<td>0.057</td>
<td>0.368</td>
</tr>
</tbody>
</table>

* R indicates positive or negative correlation
** P-value indicates strength of correlation.

* Mean significant correlation. ** Mean highly significant correlation.
Both TNF-α and IL-6 have highly significant correlation with age, blood glucose, urea, creatinine, duration of hypertension and SBP. No correlation with gender, BMI and DBP (table 2, and 3).

**Table (2): correlation between IL6 in pg/ml and study variables.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>R-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>0.001</td>
<td>0.984</td>
</tr>
<tr>
<td>Age in years</td>
<td>0.252</td>
<td>*0.000</td>
</tr>
<tr>
<td>FBG in mg/dl</td>
<td>0.199</td>
<td>0.002</td>
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<tr>
<td>Urea in mg/dl</td>
<td>0.633</td>
<td>0.000</td>
</tr>
<tr>
<td>Creatinine in mg/dl</td>
<td>0.779</td>
<td>0.000</td>
</tr>
<tr>
<td>Duration in year</td>
<td>0.371</td>
<td>0.000</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.006</td>
<td>0.923</td>
</tr>
<tr>
<td>SBP</td>
<td>0.193</td>
<td>**0.002</td>
</tr>
<tr>
<td>DBP</td>
<td>0.077</td>
<td>0.225</td>
</tr>
</tbody>
</table>

*R* indicates positive or negative correlation *P*-value indicates strength of correlation.

* Mean significant correlation. ** Mean highly significant correlation.

**DISCUSSION**

The result of present study provides experimental evidence that, significant increase in MCR, IL-6 and TNF-α was observed in essential hypertensive patients when compared with control group with (P-value 0.000, 0.000, 0.000) respectively. The relation between MCR and hypertension was in agreement with Glasscock RJ (2006) who claimed that there is positive link between high blood pressure and microalbuminuria. High blood pressure may cause microalbuminuria by increasing glomerular filtration pressure and subsequent renal damage. Some of the study showed approximately 30% of hypertensive patients had
microalbuminuria positive and some other studies reported even higher prevalence of microalbuminuria in hypertensive patients.\textsuperscript{[23]} In addition, population-based study shows a significant association between IL-6 and TNF-\(\alpha\), two markers of chronic mild inflammation, and the presence of HBP among apparently healthy subjects. The prevalence of HBP increased significantly with increased level of IL-6.\textsuperscript{[24]} Some reports.\textsuperscript{[25-27]} have shown a significant association between inflammatory markers and elevated BP, consistent with our findings.

There was positive correlation between MCR and study variables. In which there were a highly significant correlation between MCR and gender, age, blood glucose, urea, creatinine and duration of hypertension, with (P-value 0.000, 0.000, 0.005, 0.000, 0.000, 0.007 and 0.000) respectively. While no correlation with others variables. In association between MCR and gender, some study reported that the prevalence of microalbuminuria in female hypertensive population and male hypertensive population were 58.7 and 46.67%, respectively. There were positive correlation between MCR and age, similar results have been reported by Bibek \textit{et al} (2012), who reported that higher level of urinary ACR was noted with the advances of age (\(P\)-value <0.001).\textsuperscript{[28]} The correlation between duration of hypertension and MCR was a significant correlation. These remarks are in line with the observation of Hitha \textit{et al} (2008), who claimed that microalbuminuria was significantly higher in those with longer duration and greater severity of hypertension (p < 0.001 in each). The study also represents positive correlation between TNF-\(\alpha\) and IL-6 in pg/ml with age, blood glucose, urea, creatinine, duration of hypertension and SBP with. While no correlation with others variables. The correlation between IL-6 and age was significant and this in agreement with Ershler \textit{WB et al} (2000), who postulated that IL-6 is a potent mediator of inflammatory processes, and it has been proposed that the age-associated increase in IL-6 accounts for certain of the phenotypic changes of advanced age, particularly those that resemble chronic inflammatory disease. Furthermore, the age-associated rise in IL-6 has been linked to lympho-proliferative disorders, multiple myeloma, osteoporosis, and Alzheimer's disease.\textsuperscript{[19]} Chronic low-grade inflammation commonly occurs with aging and this has been termed “inflammaging”\textsuperscript{[30]} Inflammaging is characterized by an imbalance of pro-inflammatory markers and anti-inflammatory markers. Levels of pro-inflammatory markers such as IL-6, TNF-\(\alpha\) and CRP are elevated, while anti-inflammatory cytokines such as interleukin-10 are reduced.\textsuperscript{[31]}
CONCLUSION
This study concludes that TNF-alpha and IL-6 are remarkably increased in hypertensive patients and may play an important role in the pathogenesis and the development of renal damage in hypertensive patients and useful for early detection of renal disease.

REFERENCES
1. Naish, Jeannette; Court, Denise Syndercombe., 2014; (2): 562.
