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Elevated Circulatory Concentrations of Proinflammatory Cytokines in Hypertensive Patients with or Without Diabetes Mellitus

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Abstract

A number of studies suggest the association between proinflammatory cytokines and inflammation/ endothelial dysfunction in patients with hypertension. In the present study we made an attempt to measure the levels of circulatory proinflammatory cytokines such as IL-6, IL-12, TNFα and IL-4 in hypertensive patients with or without type 2 diabetes mellitus (T2DM). A total of 81 (M/F: 33/48) subjects with or without hypertension were enrolled for this study. Group 1 were normotensive, group 2 hypertensive and group 3 were hypertensive T2DM patients. Our study revealed significantly increased circulatory IL-1β, IL-6, IL-12 and TNFα levels in hypertensive subjects as compared to the healthy volunteers. However, there was no significant difference between hypertensive T2DM patients and other hypertensive subjects. This data suggests that the augmented levels of these proinflammatory cytokines is a common factor in the pathogenesis of both hypertension and hypertension associated with T2DM.

Keywords: Cytokines, Diabetes mellitus, Hypertension, Inflammation.

Introduction

Hypertension (high blood pressure) is a common cause of cardiovascular disease (CVD) which affects approximately one-third of the adult population [1]. In the past several decades various epidemiological studies suggest the interrelationship between hypertension and the enhanced CVD risk [2]. Hypertension research has progressed extensively but the exact mechanism of blood pressure elevation is yet to be unraveled.

Inflammation is a preventive measure against injury or infection. It involves the identifying of the affected tissue, leukocyte recruitment into the tissue, elimination of the offending agent and repair. Oxidative stress, overactivity of renin angiotensin aldosterone system (RAAS), and inflammation can lead to elevation in blood pressure. Enhanced blood pressure is not only due to sympathetic stimuli but also due to hormones such as angiotensin II and aldosterone. Overall this leads to modification in the proteins, and these altered proteins are recognized as non-self by the immune system, which can stimulate T cells to secrete proinflammatory cytokines (PIC's) leading to a vicious cycle of

inflammation [3][4]. Adipose tissue is an active secretory organ that secretes a number of proteins, mainly the adipocytokines, proinflammatory molecules, leptin, adiponectin, visfatin and resistin that modulate metabolic changes in hypertension. Adipose tissue is also a strong amplifier of insulin resistance (IR). Both hypertension and T2DM represent risk factors for CVD, however, the association between these vital risk factors in the pathogenesis of CVD has not yet been unraveled. It involves a variety of factors, but recent reports suggest that low-grade inflammation could be the underlying cause [5][6].

In order to clarify the possibilities of a direct pathogenic effect of proinflammatory cytokines in altering the mechanisms of the onset of high blood pressure, we evaluated the circulatory concentrations of some proinflammatory cytokines in hypertensive patients, with or without T2DM. This would help us to correlate the risk between the various cytokines and the severity of hypertension.

Methods

Subjects and Study design

We performed a cross-sectional study with 81 subjects having an average of 53±10.6 years. The patients were recruited from the Rajah Muthiah Medical College, Annamalai University, Tamil Nadu, India from May 2017- December 2017. The study was approved by ethics review committee of Annamalai University (IHEC/0189/2017). The outpatient subjects, invited to the hospital were interviewed about medical history and current medications. Complications and current health status of subjects were recorded. Informed consent was obtained from all the patients and controls. They were divided into three groups: **group 1** were normotensive subjects (n=20); **group 2** were hypertensive subjects (n=44) and **group 3** were subjects with hypertension and type 2 diabetes mellitus (n=17).

Inclusion and exclusion criteria

Adult hypertensive patients under treatment with antihypertensive drugs (ACE inhibitors, angiotensin receptor blockers and/or calcium blockers) were included in the study. T2DM patients under treatment with oral glycemic agents/insulin were also included. Patients with a history of chronic infections, malignancy, hyper/hypothyroidism, kidney diseases and autoimmune diseases were excluded from the study.

Diagnosing hypertension and type 2 diabetes mellitus

Hypertension was diagnosed based on the World Health Organization criteria. Blood pressure was recorded in three separate measurements during sitting position using a standard mercury sphygmomanometer after allowing the subjects to rest for at least 10 minutes. In this study the control subjects without hypertension were not on any medications and were noted to have blood pressure of less than 140/90. The presence of obesity was determined by using BMI. For this height, weight, waist and hip circumference were measured and the BMI calculated using Quetlet`s equation. Patients having fasting plasma glucose (FPG) levels of ≥ 126 mg/dl (7.0 mmol/l) and postprandial plasma glucose levels of ≥200 mg/dl were categorized as T2DM.

Laboratory measurements

The blood samples were drawn after 12 hours overnight fast and were stored at -70 °C until assayed. All analyses were carried out during the same day. A volume of fasting venous blood sample was collected in Clot activator tube with aseptic precautions and serum were separated. We determined the plasma concentrtions of IL-1β, IL-6, IL-12 and TNFα) in all the control and study subjects using a Bio-Plex Pro™ in a multiplex bead-based assay system (BioRad, Hercules, California, USA).

Statistical analysis

All data are reported as mean ± SD for continuous variables. p values were calculated using one way ANOVA test on PRISM software (version 6.0).

Results

Table 1 shows the clinical characteristics and biochemical parameters of the study subjects. Age difference was not significant among or between the hypertensive subjects, though BMI was significantly elevated in all the hypertensive subjects (group 2 and 3) as compared to the control (group 1), there was no significant difference in the age between the hypertensive subjects: but they were comparatively older than the control subjects. Fasting plasma glucose was significantly higher in hypertensive T2DM subjects (group 3) as compared to hypertensive (group 2) and normotensive (group 1) subjects. Total cholesterol was found to be elevated in group 3 as compared to group 4 (hypertension) and group 1 (normotensive) subjects. Serum triglycerides and LDL cholesterol were also found to be significantly increased in hypertensive subjects (group 3) as well as the hypertensive T2DM patients as compared to the control.

Figure 1 shows the plasma levels of IL-1β, IL-6, IL-12 and TNF α in the study subjects. All the above cytokine levels were significantly elevated in all the hypertensive subjects (group 2 and 3) as compared to the control (group 1) ($p<0.001$). However, when IL-1β, IL-6, IL-12 and TNF α levels of hypertensive subjects (group 2) were compared with those of the diabetic hypertensive subjects (group 3) there was no significant difference in the cytokine levels.

Discussion

The risk of cardiovascular disease increase with age and especially in association with obesity, hypertension, insulin resistance (IR) and dyslipidemia. Adipose tissue is an endocrine organ that strongly amplifies IR. Enlargement of adipose tissue, enhances the number of adipose tissue macrophages, which inturn secretes a number of proinflammatory cytokines (PIC's), that are released into the circulation [7], thereby excessively augmenting inflammation. Carley et al [8] have been shown that the levels of these cytokines are reduced in patients who lost weight. Our study shows similar results wherein a direct correlation was observed between increased bodymass index (BMI) and hypertension with or without type 2 diabetes mellitus (T2DM). Total cholesterol, triglycerides and LDL also followed a similar pattern. In this context, it is known that obesity is directly associated with enhanced circulatory IL-6, which could contribute to the pathogenesis of IR [9]. Moreover IL-6 is also known to alter insulin sensitivity, [8][10] enhance lipolysis and reduce LPL activity in adipocytes. Further, a number of studies show that insulin and central obesity are known to regulate IL-6 gene expression.

Hypertension and T2DM are both important risk factors for the future development of CVD. The exact link between these vital risk factors in the pathogenesis of CVD is still not clear. Low grade inflammation, might be a possible underlying cause [9].

IL-1β is released primarily by monocytes and macrophages as well as by non-immune cells, such as fibroblasts and endothelial cells during cell injury, invasion and inflammation. Similarly, IL-6 is secreted by macrophages and by other non-immune cells which acts both as a proinflammatory cytokine and an anti-inflammatory cytokine. IL-12 is also a proinflammatory cytokine produced by dendritic cells, macrophages, neutrophils and plasma cells and is secreted in response to antigenic stimuli. TNF α known as catechin, is another inflammatory cytokine, that plays a well-established, vital role in inflammation. TNF α acts on several different signaling pathways, through two cell surface receptors

TNFR1 and TNFR2 to regulate apoptosis and inflammation.Our results show significantly elevated IL-1β, IL-6, IL-12 and TNF α in hypertensive patients as well as in hypertensive T2DM subjects.

During hypertension, T-cell derived signals promote entry of macrophages into the endothelium of blood vessel walls and kidney, which results in cytokine release. Besides the activated T-cells promote vasoconstriction and remodeling together with Na+ and H_2O retention in the kidney resulting in more severe hypertension [11][12]. Moreover, these infiltrating mononuclear cells further secrete or induce several PIC's such as IL-6, IL-1β and TNFα [13][14]. These cytokines promote the production of a number of endothelial proteins such as endothelin [15], decrease acetyl choline induced vasodilation [16]; and destabilize eNOS mRNA [17][18], which inturn reduces NOS protein expression and prevents vasodilation. Thus endothelial dysfunction associated with many forms of hypertension may in part be mediated by PIC's [19].

In addition, PIC's are reported to be associated with impairments of pulse wave velocity leading to arterial stiffness and [20][21][22]. In T2DM, increased C-reactive protein (CRP) induces the adipose tissue macrophages to secrete PIC's which in turn demonstrates prothrombotic effects, leading to endothelial dysfunction. In addition PIC's can also increase cortisol secretion, which further exemplifies hypertension. Besides, obesity associated with IR, leads to increased sympathetic activity and elevated norepinephrine levels, leading to IR [9].

Our results correlate with the above studies in that the levels of all the PIC's evaluated were significantly elevated in hypertensive T2DM subjects as compared to notmotensive subjects.

Conclusion

Overall our results demonstrate that inflammation can play a significant role in the pathogenesis of both hypertension and T2DM. Significant beneficial therapeutic effects can be achieved by targeting inflammation in hypertension and hypertension associated with T2DM.

Figure legends

Figure 1: The circulatory levels of inflammatory cytokines in the study subjects, as measured by the multiplex assay.

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Figure 1: The circulatory levels of inflammatory cytokines in the study subjects, as measured by the multiplex assay.

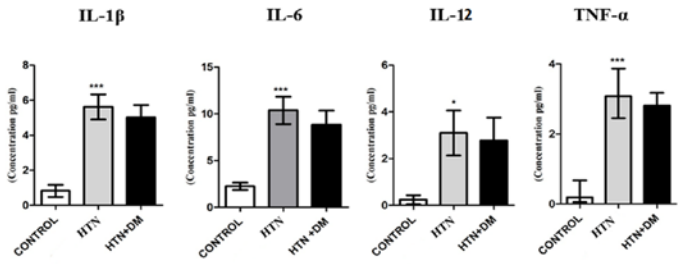

Table: 1 Demographic and clinical characteristics of the study subjects

Clinical Parameters	CONTROL	HTN	HTN+DM	One way ANOVA
Age, years	43.6 ± 9.33	54.4 ± 11.2	56.2 ± 11.5	0.002
BMI kg/m²	22.2 ± 1.35	27.0 ± 4.7	26.3 ± 3.1	< 0.0001
Systolic blood pressure, mm Hg	112.0 ± 7.6	160.0 ± 19.6	165.4 ± 15.3	< 0.0001
Diastolic blood pressure, mm Hg	78.5 ± 6.7	97.3 ± 9.9	101.8 ± 10.8	< 0.0001
Fasting plasma glucose, mg/dL	95.4 ± 6.5	103.5 ± 44.5	236.7 ± 70.5	< 0.0001
Total serum cholesterol, mg/dL	155.3 ± 26.1	172.0 ± 26.9	201.8 ± 48.8	< 0.0001
Serum triglycerides, mg/dL	103.2 ± 25.3	112.3 ± 27.1	186.3 ± 73.9	< 0.0001
HDL-cholesterol, mg/dL	45.5 ± 6.9	46.7 ± 19.4	49.1 ± 25.7	0.64
LDL-cholesterol, mg/dL	81.8 ± 9.9	107.3 ± 26.9	121.1 ± 47.2	0.0007
Urea, mg/dL	20.6 ± 3.2	28.4 ± 5.9	29.8 ± 4.3	< 0.0001
Creatinine, mg/dL	0.8 ± 0.1	0.8 ± 0.10	0.84± 0.10	0.47

All data are reported as mean ± SD for continuous variables. Unless specified. *p* values were calculated using One-way ANOVA test on PRISM software(version 6.0). BMI-Body Mass Index; HDL-High Density Lipoprotein; LDL-Low Density Lipoprotein