Revista Argentina de Clínica Psicológica
2021, Vol. XXX, N°2, 823-828
DOI: 10.24205/03276716.2020.4087

The expression change and significance of inflammatory factors in hypertensive diseases

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Abstract

Purpose: To investigate the significance of expression change and the level change of inflammatory cytokines in hypertensive diseases.

Methods: Sixteen spontaneously hypertensive rats were selected, including 6 rats with 8 weeks old (group A) and 6 rats with 16 weeks old (group B); another 12 normal Wistar-Kyoto rats were selected, including 6 rats with 8 weeks old (group C) and 6 rats with 16 weeks old (group D). All rats were male and were fed for 8 weeks. The expression of TNF-α, IL-6, hs-CRP were detected by using ELISA method. At the same time, the blood pressure, the heart weight index and the pathological morphology of the rats were observed.

Results: The levels of IL-6[(259.11±17.88) Pg/ml], TNF-α [(21.57±1.91) Pg/m]and hs-CRP [(1.54±0.34) ng/ml] were significantly higher than the other groups, there was statistically significant difference (P<0.05) in group B; the levels of IL-6 [(201.72±28.91) Pg/ml], TNF-α [(14.33±3.15) Pg/m] and hs-CRP [(1.54±0.34) ng/ml] were significantly higher than that of group C and group D, (P<0.05). In the group A and the group B, the myocardial interstitial cells were massively proliferated, and there was a significant inflammatory infiltration.

Conclusions: With the aggravation of hypertension, the level of serum inflammatory factors was further increased, which suggests that inflammatory factors may play a role in the progression of hypertension, and the severity of hypertension is closely related to the pathological changes of heart.

Keywords: Hypertension; animal experiment; inflammatory factor; tumor necrosis factor; interleukin-6; high sensitivity C reactive protein

Introduction

As one of the most common chronic diseases, hypertension is also a risk factor for late complications of other organs and tissues [1,2], and the role of primary hypertension is more significant. A study shows that [3-4] hypertension is one of the risk factors of atherosclerosis, heart and kidney dysfunction and coronary heart disease. The damage of endothelial cell function and so on, the interaction of above methods can make the control of hypertension more difficult. Therefore, the effective control rate of hypertension is low, but there is an increase in the incidence of hypertension worldwide, in addition, severe hypertension often involves other organ diseases, making hypertension become the main disease threatening human life. The expression levels of several inflammatory factors were changed in hypertensive individuals, such as [5] vascular endothelial injury factor may play an important role in the development of hypertension; there are also studies that believes hypertension itself is a chronic inflammatory reaction. Inflammatory factor is a substance that can participate in inflammatory reaction, which is secreted by immune cells, including proinflammatory and anti-inflammatory cytokines. hs-CRP can effectively reflect the inflammatory state of the body, has a high sensitivity to mild aseptic inflammation, and has been widely used in various clinical diseases Detection of inflammation status. In addition, it has the side effects of damaging endothelial cells or causing vascular dysfunction. Since hypertension is closely related to vascular endothelial damage, we believe that TNF-α is related to PIH. C-reactive protein (CRP) is a protein produced by the body under stress, which can help the body resist foreign invasions, remove the garbage produced in the body, and play a

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protective role [6]. Spontaneously hypertensive rats have the advantage that are very similar to those of human primary hypertension, which are wildly applied to basic experimental research that studying on the pathogenesis of hypertension and screening of antihypertensive drugs; Wistar-Kyoto rats are commonly used in the study of hypertension compared with spontaneous hypertension model.

Analysis of previous studies in patients with hypertension of existing organ injury studied the level of inflammatory cytokines and the correlation, thus, on the basis of previous reports, this study was conducted to investigate the relationship between the changes of inflammatory factors and the development of hypertension in different developmental stages of hypertension.

Materials and methods

Experimental materials

Six Male spontaneously hypertensive rats (Shandong University) with 8 weeks old (group A) and 16 weeks old (group B) were selected in each group and six Wistar-Kyoto male rats (Shanghai Laboratory Animal Center) with 8 weeks (group C) and 16 weeks (group D) were selected in each group. A total of 24 rats were required, all rats were weighed between 200-250 G.

Rats were used for all experiments, and all procedures were approved by the Animal Ethics Committee of Qingdao Hospital of Traditional Chinese Medicine.

Methods

Reagents and experimental instruments

Reagents

①HsCRP assay kit was purchased from Sichuan Xincheng Biotechnology Co., ltd.;Rat IL-6 ELISA kit was purchased from Xiamen Huijia Biotechnology Co. ltd.; ③ Rat TNF -α detection kit, purchased from the Shanghai Lan Lan Biological Technology Co., ltd

Main test equipment

Enzyme standard analyzer was purchased from Nanjing iron Experimental Equipment Co., Ltd., model for HBS-1096B enzyme analyzer.

Experimental method

All rats were fed a normal diet, the serum IL-6, TNF-αand hsCRP were detected after 8 weeks of feeding. Specific operations are as follows: after 8 weeks of feeding, the rats were anesthetized with chloral hydrate (10g/dL) by intraperitoneal anesthesia,

5 ml specimen of obtained from abdominal aorta. The specimens were placed under the condition of 3000r/min centrifugation for 10min, then the serum was separated for further detection. The specimen collection should be completed with 30min, if it is not measured in time, the separated serum should be kept at -20 ^{o}C.

Observation content

Cardiac pathology observation

HE staining was used to stain the myocardial cells, the infiltration of inflammatory cells was observed under microscope.

Inflammatory factor

All indexes were detected by double sandwich enzyme-linked immunosorbent assay (ELISA), the test procedure is carried out strictly in accordance with the kit instructions.

Statistical processing

All data were analyzed using SPSS software. The data is recorded in the form of mean ± standard deviation, T-test was used to compare the differences, and the difference was statistically significant in $P<0.05$. The linear correlation analysis of each index was made by using EXCEL software.

Results

Results of hypertension model

Twenty-four rats were fed for 8 weeks, 12 Wistar-Kyoto male rats of different ages were kept healthy, inflammatory diseases of no influence and tissue inflammation occur, that was there were neither diseases of influence the level of inflammatory nor normal blood pressure; according to reference[6], the blood pressure was evaluated in group A and group B, the results were as follows: the rats in group A had high blood pressure after feeding, the mean systolic pressure was (158 ± 11.6) mmHg; the rats in group B has been suffering from high blood pressure which mean systolic blood pressure (159 ± 9.7) mmHg, after 8 weeks of feeding, the mean systolic blood pressure was (203± 8.3) mmHg.

Results the level of inflammatory factors in rats of each group

The results show that the levels of IL-6 in group A [(259.11 ±17.88) Pg/ml], TNF-α [(21.57 ± 1.91) Pg/m], hsCRP [(1.89±0.21) ng/ml] were significantly higher than the other groups, there was statistically significant difference ($P<0.05$); The level of IL-6[(201.72±28.91) Pg/ml], TNF-α [(14.33±3.15) Pg/m] and hsCRP [(1.54±0.34) ng/ml] in group A1 (Table 1).

At the same time, the relationship between the

level of inflammatory factors and the severity of hypertension was analyzed, with the aggravation of hypertension, the level of inflammatory factors increased linearly, the linear equation is analyzed as follows: ①IL and hypertension: y = -63.57x + 324.74，R^2 = 0.9969；②TNF and hypertension: y = -5.12x + 25.983，R^2 = 0.9459；③hsCRP and hypertension: y = -0.42x + 2.3333，R^2 = 0.9908；(Figure 1.)

Pathological observation of cardiac tissue

HE staining showed that the thickness of 3μm in myocardial tissue was displayed: the proliferation of myocardial interstitial cells was active, and there was a significant inflammatory infiltration in the interstitium, (Figure 2).

Discussion

Hypertension is divided into primary hypertension and secondary hypertension, secondary hypertension is a complication of other diseases, but the pathogenesis of primary hypertension has not been clear, it is generally believed that it is closely related to heredity, obesity, diet, emergency and other factors [7-9], which is the result of multiple factors. In recent years, a number of studies have shown that inflammatory factors play an important role in the progression of essential hypertension [10-12]. On the basis of this study, the aim of this study was to investigate the relationship between inflammatory factors and hypertension in spontaneously hypertensive rats. In this study, we chose spontaneously hypertensive rats as the model of hypertension, in addition to the high incidence of hypertension in spontaneously hypertensive rats (100%), the main reason for this may be that polygenic inheritance may determine elevated blood pressure in spontaneously hypertensive rats, this feature is similar to that of human primary hypertension, therefore, the animal model of spontaneously hypertensive rats is regarded as the ideal model of hypertension pathogenesis and screening antihypertensive drugs. The clinical features of hypertension in spontaneously hypertensive rats are similar to those of human primary hypertension: there was no obvious organic change in the early stage of hypertension; the blood pressure increased with the increase of the age of the rats, and rose to the highest level in 6 months; the total peripheral resistance of blood vessels was significantly increased and the hemodynamic changes were consistent; the complications such as heart, brain, kidney and so on may occur in the later period of hypertension, and the therapeutic measures such as antihypertensive drugs can prevent or reduce the progression of the disease and the occurrence of complications; stress and excessive intake of salt and other factors can accelerate the development of hypertension and aggravate complications.

Therefore, our study chooses the spontaneous hypertensive rats as the research object of hypertension. At the same time, this study used different weeks old spontaneously hypertensive rats to build different stages of the development of hypertension model, the severity of the 16-week-old spontaneously hypertensive rats was much higher than that of the 8-week-old group, it can be used to observe the changes of inflammatory factors in different stages of hypertension.

The results of this study showed that the level of IL -6 [(259.11±17.88) Pg/ml] Pg/ml] in group B was significantly higher than that in other groups, the difference was statistically significant (P<0.05); the level of IL-6[(201.72±28.91) Pg/ml]in group A1 (Table 1). The results were similar to those of significant increase of TNF and IL-6 in serum in patients with primary hypertension [13-14]. Interleukin -6 is a pleiotropic cytokine secreted by T cells and endothelial cells, which main function is inflammation and cell protection, at the same time, it can also stimulate the production of [15-16] hsCRP. The results suggest that the inflammatory response occurs in the subjects with hypertension, which leads to the increase of IL -6 level; however, the imbalance of the secretion of IL-6 also indicates that the endothelial function in hypertensive subjects is disordered, it may be the mechanism by which peripheral vascular resistance increases and promotes hypertension. In addition, there are studies [17] show that IL-6 have the ability of promoting smooth muscle proliferation and increasing the concentration of intracellular calcium ions, the blood vessels strongly contract so that blood pressure increase. The experiment also showed that the TNF-a [(21.57±1.91) Pg/m], hsCRP[(1.89±0.21) ng/ml] level in group B were significantly higher than those in other groups, the difference was statistically significant (P<0.05);TNF-a[(14.33±3.15)Pg/m],hsCRP[(1.54±0.34)ng/ml] level in group A1 (Table 1). It is similar with the level increase of hsCRP in different research objective with hypertension [18-19] and the significant level increase of TNF-a and IL-6 in different research objective with hypertension [20-21]. The study also showed that high sensitivity C reactive protein has a certain predictive effect on the progression of hypertension [22]. When the concentration of hs

CRP is too high; it can inhibit the release of NO and indirectly promote atherosclerosis and hypertension. CRP is the most sensitive indicator of the body's inflammatory response, and has a certain impact on the occurrence and development of various medical diseases such as hypertension, diabetes, stem cells, and hyperlipidemia. Research shows [23] that TNF plays an important role in the regulation of cardiovascular function. Animal experiments showed that [24] the activation of TNF system was closely related to blood pressure. In our study the rats in group B become Spontaneously hypertensive rats after being feeding 24 weeks, in theory, the level of blood pressure has reached the peak, the most serious hypertension, the level of blood pressure has reached the peak, which is the most serious illness of hypertension, our study also found that the inflammatory indexes in this group had the highest expression. This shows that the level of inflammatory factors and the severity of hypertension have a certain relationship. This suggests that inflammatory factors and hypertension may promote each other development. The level of inflammatory factors directly or indirectly led to the occurrence of vascular endothelial dysfunction or abnormal blood pressure caused by high blood pressure; after hypertension occurrence and further further inflammation occurred caused by abnormal levels of inflammatory factors. At the same time, we performed a simple linear analysis of inflammatory factors and hypertension, the result found that both are a positive correlation, the higher the severity of hypertension, the higher the expression of inflammatory cytokines in serum. At the time of data processing, the data of group C and group D without hypertension were taken as the average value, so the linear graph presented the relationship as normal, mild hypertension and moderate / severe hypertension. At the same time, we observed the pathological changes of heart tissue in rats, the results showed that the interstitial cells of myocardial tissue in group A and group B were proliferated, there was a significant inflammatory cell infiltration, but in the group C and group D did not this phenomenon. It also proves that hypertension is closely related to the pathological changes of the heart, which is an important factor of myocardial hypertrophy. However, this study is mainly based on the expression of inflammatory factors in hypertensive rats to analyze both relationships, therefore, the study may have some limitations. To study the mechanism of inflammatory factors in the promotion of hypertension, other mechanisms are needed.

To sum up, in animal studies, the level of serum inflammatory factors in hypertensive rats was significantly higher than those without hypertension; with the aggravation of hypertension, the level of serum inflammatory factors was further increased, this suggests that inflammatory factors may play a role in the progression of hypertension, and the severity of hypertension is closely related to the pathological changes of heart.

Disclosure of conflict of interest

None.

References

[1] Reuter, S., et al., Hypertension. Acta Physiol (Oxf), 2014 ,211(1): p.1–4.
[2] Guignabert, C., et al., Restoring BMPRII Functions in Pulmonary Arterial Hypertension: Opportunities, challenges and limitations. Expert opin ther targets,2016, 28: p.1-10.
[3] Chen.Z., et al., H-type hypertension is an important risk factor of carotid atherosclerotic plaques. Clinical and Experimental Hypertension,2016, 38(5): p.424-8.
[4] Oliveras, V., et al., The relevance of the ankle-arm index to the reclassification of cardiovascular risk in asymptomatic hypertensive middle-aged men. Medicina Clínica, 2015 May, 144(10): p.435-9.
[5] Gu, P., et al., Elevating circulation chemerin level is associated with endothelial dysfunction and early atherosclerotic changes in essential hypertensive patients. J Hypertens,2015,33(8): p.1624-32.
[6] Kong, Dehua et al., Correlation between the expression of inflammatory cytokines IL-6, TNF-α and hs-CRP and unfavorable fetal outcomes in patients with pregnancy-induced hypertension. Experimental and therapeutic medicine 2018:1982-1986.
[7] Koudstaal, T et al., Plasma markers in pulmonary hypertension subgroups correlate with patient survival. Respiratory research, 2021, 22(1):137.
[8] Guo, L., et al., Acupoint massage nursing conducive to improve curative effect of the obesity patients who are complicated with hypertension and are treated by oral drugs. Int J Cli Exp Med, 2015, 8(7): p.11727-33.
[9] Dalai, N., et al., Risk factors for the development of essential hypertension in a Mongolian population of China: a case-control study. Genet Mol Res, 2014, 13(2): p.3283-3291.

[10] Athanase, D., et al., Arterial Stiffness and Incidence of Systolic Hypertension: The End to the "Chicken-Egg" Question? J Cli Hypertens (Greenwich),2015, 17(8): p.592–3.
[11] Chen, Y., et al., [Relationships between insulin resistant and hypertension and traditional Chinese medicine intervention]. Journal of Chinese Integrative Medicine, 2010 ,8(12): p.1114-8.
[12] Ansarin, K., et al., Echocardiographic Evaluation of the relationship Between inflammatory factors (IL6, TNFα, hs-CRP) and Secondary Pulmonary Hypertension in patients with COPD.A Cross sectional study. Pneumologia, 2015, 64(3): p.31-5.
[13] Jian, L., et al., Functional analysis of UMOD gene and its effect on inflammatory cytokines in serum of essential hypertension patients. International Journal of Clinical & Experimental Pathology, 2015, 8(9) :P.11356-63.
[14] Shang, Y., et al., Clinical significance of interleukin-6, tumor necrosis factor-α and high-sensitivity C-reactive protein in neonates with hypoxic-ischemic encephalopathy. Experimental & Therapeutic Medicine, 2014,8(8) :P.1259-1262.
[15] Ura, N., et al., Serum Interleukin-6 Levels in the Differential Diagnosis of Sepsis and Transient Tachypnea of Newborn. Medical Journal of Trakya University, 2010, 27(3) :P.363-366.
[16] Jin, J.Y., et al., Value of procalcitonin and interleukin-6 in diagnosis of early infections and prognosis of patients with acute pancreatitis. Chinese Journal of Nosocomiology, 2012, 22(19): p.4258-4260.
[17] Liang, M., et al., Paeonol Inhibits the Proliferation, Invasion, and Inflammatory Reaction Induced by TNF-α in Vascular Smooth Muscle Cells. Cell Biochemistry and Biophysics, 2015, 73(2) :P.1-9.
[18] Winata., et al., Comparing Albuminuria and High Sensitivity C-Reactive Protein as Predictors of Silent Myocardial Ischemia in Hypertensive Men without Diabetes Mellitus. Journal of Hypertension, 2015, 33: p.e11.
[19] Guzel, S., et al., Osteoprotegerin, leptin and IL-6: association with silent myocardial ischemia in type 2 diabetes mellitus. Diabetes & Vascular Disease Research Official Journal of the International Society of Diabetes & Vascular Disease, 2013, 10(1) :P.25.
[20] Lee, B.T., et al. Association of C-reactive protein, tumor necrosis factor-alpha, and interleukin-6 with chronic kidney disease. BMC Nephrol, 2015, 16: p.77.
[21] Chen, H., et al., The clinical significance of serum inflammatory cytokines TNF-α, IL-6 and hs-CRP in essential hypertension patients. Shanxi Medical Journal, 2015, 33(19): P.1021-1024.
[22] Lee, J.H., et al., Incremental predictive value of high-sensitivity C-reactive protein for incident hypertension: the Hypertension-Diabetes Daegu Initiative study. Clin Exp Hypertens, 2014, 36(5) :P.302-8.
[23] Yi, W., et al., C1q/tumor necrosis factor-related protein-3, a newly identified adipokine, is a novel antiapoptotic, proangiogenic, and cardioprotective molecule in the ischemic mouse heart. Circulation, 2012,125(25): p.3159-69.
[24] Omote, Y., et al., Telmisartan Promotes Potential Glucose Homeo -stasis in Stroke-Resistant Spontaneously Hypertensive Rats via Peroxisome Proliferator-Activated Receptor γ Activation. Curr Neurovasc Res, 2015, 12(1): p.91-97.

Tables and Figure legends

Table 1. **The detection results of the level of inflammatory factors in each group**

group	N	IL-6(Pg/ml)	TNF-α(Pg/ml)	Hs CRP (ng/ml)
A	6	201.72±28.91*	14.33±3.15*	1.54±0.34*
B	6	259.11±17.88*#	21.57±1.91*#	1.89±0.21*#
C	6	129.36±17.43	10.72±2.88	1.01±0.17
D	6	131.97±21.35	11.93±1.33	1.09±0.23

Note: group A, group A1 compared with group B, group B1, the difference was statistically significant different, *P<0.05; group A compared with group A1, the difference was statistically significant different, *P<0.05.

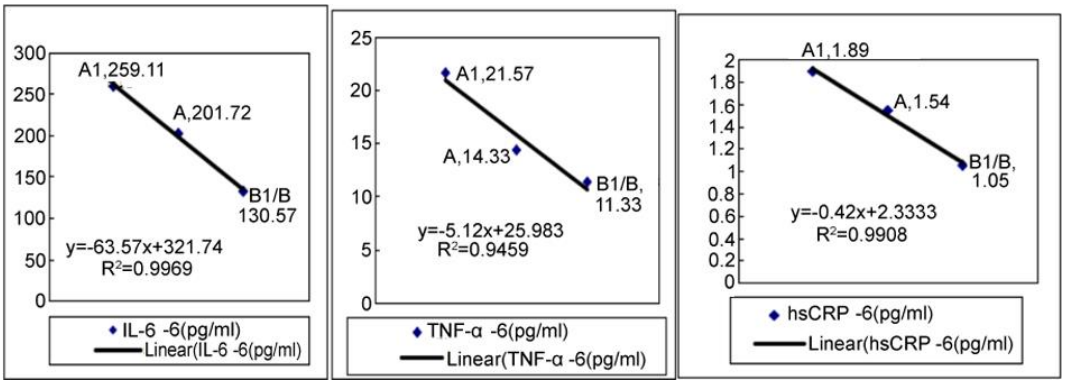

Figure 1. **Linear analysis between the levels of inflammatory factors and hypertension.**

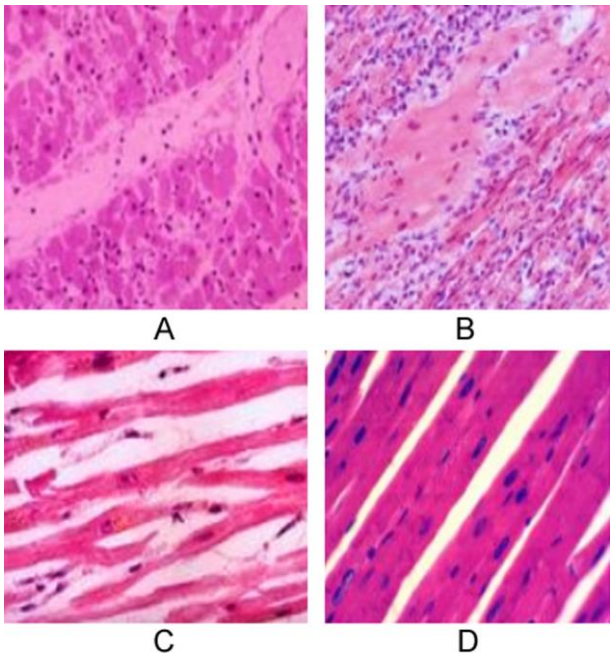

Figure 2. **Pathological observation of cardiac tissue of rats in each group**

Note: Figure 2-A showed rare inflammatory factors, Figure 2-b showed a large number inflammatory factors infiltration; Figure 2-c and Figure 2-d showed no inflammatory factors.