

## Original Article

# Patients with metabolic syndrome have higher single tlr4 positive rate of blood mononuclear cells compared with simple hypertension patients

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Received November 22, 2015; Accepted April 12, 2016; Epub June 15, 2016; Published June 30, 2016

**Abstract:** Objective: To compare the expression of TLR4 in blood mononuclear cells and plasma high-sensitivity C-reactive (hsCRP) of metabolic syndrome (MS) patients and simple hypertensive patients. Methods: Flow cytometry was used to detect TLR4 positive rate of blood mononuclear cells and plasma hsCRP concentrations of 62 EH patients and 45 patients with MS. TLR4 and hsCRP were associated with age, sex, cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), fasting blood glucose (FBG) and glycosylated hemoglobin (HbA1c), TLR4 and hsCRP to conduct univariate analysis and multiple stepwise regression analysis. Results: TLR4 was  $(5.7 \pm 1.2)\%$  and hsCRP was  $(5.6 \pm 0.9)$  mg/L in MS group, while  $(5.3 \pm 0.9)\%$  and  $(5.0 \pm 0.8)$  mg/L in EH group. TLR4 and hsCRP of these two groups were statistically significant (*t* values were -3.274, -2.225, *P* values were 0.028, 0.01); Univariate analysis showed that TLR4 had positive correlation with TC, LDL, HbA1c, FBG, PBG and hsCRP ( $P < 0.05$ ), and hsCRP was positively correlated with TC, LDL, HbA1c, FBG, PBG, TLR4 ( $P < 0.05$ ); Meanwhile, multiple stepwise regression analysis showed that hsCRP and HbA1c were the most significant factors which affect TLR4 ( $\beta$  values were 0.745, 0.244, *P* values were 0.000, 0.004), and TLR4 was the most significant factor which affect hsCRP ( $\beta = 0.943$ ,  $P = 0.000$ ). Conclusion: It was MS patients that had higher single TLR4 positive rate of mononuclear cells and plasma hsCRP compared with EH patients. TLR4 positive rate and plasma hsCRP levels of MS patients were positively correlated. And TLR4 may be involved in the development and progression of MS.

**Keywords:** Metabolic syndrome, single tlr4, hypertension

## Introduction

Metabolic syndrome (metabolic syndrome, MS) has become one of the major diseases of modern society which can be harmful to human health [1]. Studies have shown that the risk of cardiovascular and cerebrovascular events in patients with metabolic syndrome is significantly greater than simple hypertension (essential hypertension, EH) and diabetic patients, which is harmful and difficult to control [2]. Its pathogenesis is complex and is not entirely clear. Toll-like receptor 4 (Toll-like receptor 4, TLR4) is a natural immune-mediated transmembrane signaling receptor, which plays an important role in signal transduction of inflammatory cell activation, and is the bridge between natural immunity and acquired immunity [3]. This paper compares TLR4's features of blood mononuclear cells of HE patients who combined with MS and

simple HE patients, and reveals different characteristics of HE patients who combined with MS and simple HE patients, from the perspective of inflammation.

## Materials and methods

### Object

Objects were 107 cases of outpatient or inpatient patients newly diagnosed with essential hypertension from cardiology department of Xuzhou Medical College and Xuzhou Mining Bureau General Hospital from 2013-07 to 2014-01. There were 45 cases of MS patients involved for metabolic syndrome group (MS group); and 62 cases of EH patients for hypertension alone group (EH group). EH patients were consistent with the diagnosis of "2010 Chinese Hypertension Prevention Guide" [4],

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**Table 1.** Patients' general situation comparison

Project	EH group	MS group	t/Z value	P value
Age (year)	49.8 ± 6.2	50.6 ± 6.6	-0.592	0.555
Gender (male/female)	38/24	26/19	-0.364*	0.716
WC (cm)	94.5 ± 7.1	82.8 ± 3.9	-11.182	0.000
BMI (kg/m <sup>2</sup> )	28.3 ± 2.9	23.8 ± 1.8	-10.044	0.000
TC (mmol/L)	4.9 ± 0.7	6.6 ± 0.6	-12.236	0.000
TG (mmol/L)	1.3 ± 0.2	2.3 ± 1.2	-5.813	0.000
LDL (mmol/L)	3.3 ± 0.8	4.4 ± 0.6	-8.911	0.000
HDL (mmol/L)	1.6 ± 0.4	1.0 ± 0.3	9.976	0.000
HbA1c (%)	5.0 ± 0.3	5.8 ± 0.8	-7.313	0.000
FBG (mmol/L)	5.1 ± 0.6	6.2 ± 1.1	-6.725	0.000
PBG (mmol/L)	6.6 ± 0.6	9.1 ± 2.4	-7.852	0.000
SBP (mmHg)	154.7 ± 9.2	157.4 ± 9.7	-1.507	0.135
DBP (mmHg)	94.8 ± 5.8	96.7 ± 7.1	-1.523	0.131

Note: \*indicates Z values, I am t values.

MS diagnosis in 2007, "Chinese adult dyslipidemia Prevention Guide" [5]. Patients General Information in **Table 1**. All patients were newly diagnosed, without taking any antihypertensive, lipid lowering, hypoglycemic drugs. Through medical history and laboratory tests to exclude secondary hypertension, heart failure, acute infectious diseases, connective tissue disease, cancer, kidney disease, coronary heart disease and so on.

### Experimental methods

**Specimen collection:** Eligible patients were tested systolic blood pressure (SBP), diastolic blood pressure (DBP), body mass index (BMI = weight/height<sup>2</sup>), waist circumference (WC) in empty stomach, and collected 15 ml of elbow venous blood for cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), fasting blood glucose (FBG), glycated hemoglobin (HbA1c), TLR4, high-sensitivity CRP (hsCRP) test, then oral 75 g glucose powder dissolved in water, and collected 5 ml of venous blood 2 hs later to measure 2 h glucose (PBG).

**Blood mononuclear cells TLR4 detect:** Anti-coagulated whole blood 150 uL, added the red cell lysate, mixed 10 min, centrifuged. Wash cells twice with 1 mL PBS, and each tube was added 20 uL PE labeled mouse anti-human monoclonal antibody of TLR4 (USA, eBioscience Corporation, purchased from Beijing Zhongshan Biotech Companies). Then incubated in dark at

room temperature after mixing 30 min; and washed once with PBS to centrifugal precipitate fluorescence staining cells. Added paraformaldehyde in each tube to fix 10 min, placed in temperature 4°C. Preservation solution was discarded by centrifugation, PBS was used to adjust cell density to 2 × 10<sup>9</sup>/L, and used flow cytometry to detect. Becton Dickinson FACSCalibur flow cytometer was used, and obtained 10,000 cells each tube.

**Glucose, lipids, hsCRP, HbA1c detect:** Use Calendar 7600 automatic biochemical analyzer, reagent for CENTRONIC-JMBH Company. Fasting peripheral venous blood of patients to test lipids, blood glucose, glycosylated hemoglobin levels with biochemical routine.

### Statistical analysis

Use SPSS17.0 software for data analysis, measure data with the mean ± standard deviation (± s), use t test for comparison between, use  $\chi^2$  test for count data, use Pearson correlation analysis for relationship between indicators of normal data, use Spearman correlation analysis for non-positive state distribution data, and use multivariate linear regression analysis for regression analysis between data. P < 0.05 is considered statistically significant.

## Results

### Patients' general situation comparison

Age, sex, SBP, DBP were not statistically different between two groups of patients (**Table 1**). BMI, TG, TC, LDL, HDL, HbA1c, FBG, PBG were statistically significant between two groups of patients (**Table 1**).

### TLR4 and hsCRP comparison and TLR4 and hsCRP correlation test results of two groups of patients

Monocytes TLR4 positive rate and plasma hsCRP of EH group were higher than MS group (t values were -3.274, -2.225, P values were 0.028, 0.01); TLR4 and hsCRP of MS group were correlated (r = 0.943, P = 0.000); TLR4

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**Table 2.** TLR4 and hsCRP comparison and TLR4 and hsCRP correlation test results of two groups of patients

	EH group	MS group
TLR (4%)	5.3 ± 0.9	5.7 ± 1.2
hsCRP (mg/L)	5.0 ± 0.8	5.6 ± 0.9
Pearson correlation analysis results	R = 0.261	R = 0.943
	P = 0.04	P = 0.000

**Table 3.** TLR4 and hsCRP correlation analysis results with each clinical index of MS group

Program	Statistics	TLR4	hsCRP
Gender*	R value	0.262	0.289
	P value	0.082	0.054
Age	r value	0.099	0.046
	P value	0.516	0.765
WC	r value	-0.123	-0.140
	P value	0.422	0.359
BMI	r value	-0.035	-0.079
	P value	0.820	0.608
TC	r value	0.309	0.300
	P value	0.039	0.046
TG	r value	0.269	0.222
	P value	0.074	0.143
LDL	r value	0.349	0.365
	P value	0.019	0.014
HDL	r value	-0.106	-0.102
	P value	0.488	0.506
HbA1c	r value	0.848	0.811
	P value	0.000	0.000
FBG	r value	0.662	0.608
	P value	0.000	0.000
PBG	r value	0.703	0.700
	P value	0.000	0.000
SBP	r value	-0.136	-0.071
	P value	0.372	0.643
DBP	r value	-0.014	0.086
	P value	0.929	0.576
TLR4	r value	#	0.943
	P value	#	0.000

Note: \*indicates using spearman correlation analysis, and the rest using Pearson correlation analysis; #indicates no data.

and hsCRP of EH group were also correlated ( $r = 0.261$ ,  $P = 0.04$ ) (Table 2).

### TLR4 and hsCRP correlation analysis results with each clinical index

TLR4 was positively correlated with TC, LDL, HbA1c, FBG, PBG, hsCRP ( $P < 0.05$ ); hsCRP

was positively correlated with TC, LDL, HbA1c, FBG, PBG, TLR4 ( $P < 0.05$ ) (Table 3).

### TLR4 and hsCRP stepwise regression analysis results with each clinical index

Did multivariate stepwise regression analysis with TLR4 as dependent variables, and age, BMI, TG, TC, LDL, HDL, HbA1c, FBG, PBG, SBP, DBP, hsCRP as independent variables, hsCRP and HbA1c entered into regression equation ( $\beta$  values were 0.745, 0.244,  $P$  values were 0.000, 0.004), the constant term was zero.

Did multivariate stepwise regression analysis with hsCRP as dependent variables, and age, BMI, TG, TC, LDL, HDL, HbA1c, FBG, PBG, SBP, DBP, TLR4 as independent variables, only TLR4 finally entered into regression equation ( $\beta = 0.943$ ,  $P = 0.000$ ), the constant term was zero (Table 3).

## Discussion

MS is a high risk factor of cardiovascular disease, which is one of the main causes of disability and death. In recent years, with increasing prevalence rate of MS, studies about MS are valued by various countries' scholars. MS pathophysiological mechanism is very complex, mainly for the aggregation of multiple risk factors. At present, from the perspective of etiology, pathogenesis of MS may have: ① insulin resistance (IR) [6, 7]; ② obesity and fat distribution abnormalities: abdominal obesity and lipid toxicity effects [8]; ③ aggregation of other independent risk factors (including oxidative stress, hormones, heredity and environment, etc.) [9]. In recent years, the relationship between chronic inflammation and MS is valued gradually [10, 11].

The results of this study showed that MS patients had higher hsCRP than simple EH patients, indicating that MS patients had higher inflammatory state compared with EH patients. Studies have shown that chronic inflammation status in vivo is the cause of MS, which promotes the occurrence of cardiovascular events in patients with MS [12].

Toll like receptor (TLR) belongs to type I transmembrane receptor family and is first discovered in *Drosophila* [13], which plays a key role in innate immune system. It not only can direct-

ly identified with specific molecular structure of certain pathogens and their products, but also can do recognition and binding of different pathogen-associated molecular pattern and trigger a series of signal transduction, leading to the release of large amounts of inflammatory mediators [14, 15]. TLR4 is one kind which studied more. In this experiment, MS patients had higher monocytes TLR4 positive rate than EH patients, and TLR4 was correlated with hsCRP of MS patients, the correlation coefficient  $r = 0.943$ , stepwise multiple regression analysis showed that with hsCRP as dependent variables, only TLR4 entered into regression equation, which indicated that increased inflammation in MS patients may be related to increased expression of TLR4. While for EH group, although there was correlation between TLR4 and hsCRP, the correlation coefficient  $r = 0.261$ , the relationship was not very close. Experiments have shown that reduce inflammatory signaling pathway by knockout mice experiments can reduce obesity-related insulin resistance [16, 17]. Besides, expression of fat TLR4 increases with obesity, and can be produced by LPS-induced NF- $\kappa$ B activation and cytokine [11]. Therefore, TLR4 may be involved in many etiological factors mentioned above which lead to pathogenesis of MS, TLR4 may be one of the connection points. As present, studies have shown that the improvement of different antihypertensive drugs for inflammation is not the same. This may be one of the reasons which cause differences in clinical cardiovascular end point events with different antihypertensive drugs in the same case. In the experiment, we did multivariate stepwise regression analysis with TLR4 as dependent variables, hsCRP and HbA1c entered into regression equation, which indicated that compared with blood fat and blood pressure, average blood glucose and had larger effects on TLR4, and insulin resistance was the main reason of abnormal blood glucose level in patients with MS, which further confirmed the core status of insulin resistance in the pathogenesis of MS.

The experiments above prove that TLR4 may mediate occurrence and development of MS. Clinical use of antihypertensive drugs should be individualized, Judge EH patients with metabolic disorders, screen related inflammatory factors, chose antihypertensive drugs reason-

ably, and develop other effects besides antihypertensive, such as anti inflammation, endothelial improvement and so on, which will help to further improve the prognosis of patients with EH.

### Disclosure of conflict of interest

None.

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