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RESEARCH ARTICLE

The Different Concentrations of Transforming Growth Factor-β1 (TGF-β1), Matrix Metalloproitenase-9 (MMP-9) and Vascular Endothelial Growth Factor Receptor-2 (VEGFR-2) in Normoalbuminuria Normotension, Normoalbuminuria Hypertension, and Microalbuminuria Hypertension

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Abstract

BACKGROUND: Vascular remodeling was an adaptive process of the vascular wall that occurred in response to long-term changes in hemodynamic conditions that contribute to the changes of the vascular structure and the pathophysiology of vascular disease.

On the other hand, Endothelial Progenitor Cells (EPC) derived from bone marrow had the capacity to migrate to the peripheral circulation and to differentiate into mature endothelial cells. Therefore, EPC could contribute in the endothelial repairing after endothelial injury.

METHODS: This study was a cross sectional design. Analysis was done among 30 subjects with normoalbuminuria normotension, 55 subjects with normoalbuminuria hypertension and 30 subjects with microalbuminuria hypertension. TGF-β1, MMP-9 and VEGFR-2 testing were performed by ELISA method. All statistical calculations were performed using the SPSS 11.5 statistical software package. We used the Independent sample T test, Mann-Whitney, One Way Annova and Kruskal Walris to establish the difference among various biochemical measures.

RESULTS: TGF-β1 concentration was increased from normoalbuminuria normotension to normoalbuminuria hypertension and to microalbuminuria hypertension (27.63178 ± 12.97246 vs 38.61193 ± 17.09546 vs 38.73939 ± 12.63911 ng/mL). TGF-β1 concentration was higher significantly in normotension as compared to hypertension (27.63178 ± 12.97246 vs 38.65692 ± 15.58950, $p < 0.001$) and to microalbuminuria hypertension (38.73939 ± 12.63911, $p < 0.001$). MMP-9 concentration was increased in normotension to normoalbuminuria hypertension but was decreased in microalbuminuria hypertension (438.1967 ± 156.4268 vs 564.5873 ± 291.2876 vs 418.6900 ± 188.3801 ng/mL). MMP-9 concentration was higher significantly in normoalbuminuria hypertension as compared to microalbuminuria hypertension ($p = 0.028$). VEGFR-2 concentration was decreased from normotension to normoalbuminuria hypertension and to microalbuminuria hypertension (9.90552 ± 1.85158 vs 9.39561 ± 1.75413 vs 9.00506 ± 1.47173 ng/mL). VEGFR-2 concentration was higher significantly in normotension as compared to microalbuminuria hypertension ($p = 0.042$).

CONCLUSIONS: The increasing concentration level of TGF-β1 and decreasing concentration level of MMP-9 in microalbuminuria hypertension showed that the remodeling process was getting increased. The decreasing concentration level of VEGFR-2 in microalbuminuria hypertension showed that the repairing process was getting decrease.

KEYWORDS: Vascular remodeling, Vascular Repairing, TGF-β1, MMP-9, VEGFR-2.

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Introduction

Vascular remodeling was an active process of structural alteration that involves changes in at least four cellular processes as cell growth, apoptosis, inflammation and fibrosis, and was dependent on dynamic interaction between locally generated growth factors, vasoactive substance and hemodynamic stimuli. (1)

Vascular remodeling in hypertension was characterized by a thickened media, a reduced lumen, and an increase extracellular matrix. These changes were associated with endothelial dysfunction. Structural changes in hypertensive vessels were associated with increase growth factors: TGF-1β, local vasoactive substances: Ang II, matric proteins: collagen and elastin, matrix proteinases: collagenase and elastase. (2)

Microalbuminuria (MAU), which was defined as an abnormal urinary excretion of albumin between 30 and 300 mg/d, was an integrated marker of cardiovascular risk and related to the severity of target organ damage in hypertension and also for identifying patients at higher global risk (3). In this research, we divided the research subjects into three different subgroups by using albuminuria to stratify the more severe organ damage in each subgroup, whereas MAU could represent endothelial dysfunction.

TGF-β regulated the proliferation and differentiation of cells, embryonic development, wound healing and angiogenesis. TGF-β also suppressed the immune system and induces extracellular-matrix components. The overproduction of TGF-β could result in excessive depostion of extracellular matrix, scar tissue and fibrosis which finally resulted to the tissue damage (4). In this research, we used TGF-β as the marker of vascular remodelling in term of its capacity to develop fibrosis onto vascular endothelium.

Increased level of MMP-9 in hypertension played an important role in vascular remodeling and was associated with destruction of the elastic laminae of arteries and aneurysm formation. Recently, MMP-9 levels had been identified as a novel predictor of cardiovascular risk in patients with coronary artery disease and stroke (2). In this research, we used MMP-9 as the marker of vascular remodelling regarding of its capacity to formn aneurysm in vascular wall.

On the other hand, Endothelial Progenitor Cells (EPCs) from bone marrow had the capacity to migrate to the peripheral circulation and to differentiate into mature endothelial cells. EPCs were characterized by the expression of VEGFR-2 as a surface marker. The main function of EPC in circulation was to repair of the injured endothelium by substitute the endothelial damage. (5) In this research, we used VEGFR-2 to represent the repairing vascular regarding of its capacity to replace the endothelial damage or dysfunction (vascular repairing).

We hypothesized that normal homeostasis was required to make a balance between remodeling and repairing vascular process in hypertension. The objective of this research was to to determine the differences in concentrations of TGF-β1, MMP-9 and VEGFR-2 among normoalbuminuria normotension, normoalbuminuria hypertension and microalbuminuria hypertension.

Patients and Methods

Thirty (30) subjects with normoalbuminuria normotension, with the following criteria : systolic blood pressure (BP) of ≤ 139 mmHg and diastolic BP of ≤ 89 mmHg and MAU excretion of < 30 µg / mg creatinine.

Fifty five (55) subjects with normoalbuminuria hypertension, with the following criteria : systolic blood pressure (BP) of ≥ 140 mmHg and/or diastolic BP of ≥ 90 mmHg and MAU excretion of < 30 µg / mg creatinine.

Thirty (30) subjects with microalbuminuria hypertension, with the following criteria : systolic blood pressure (BP) of ≥ 140 mmHg and/or diastolic BP of ≥ 90 mmHg and MAU excretion of ≥ 30 µg / mg creatinine.

This study was a cross sectional design. All participants gave their informed consent to participate in this study.

Assays of Biochemical Markers

Venous blood was collected from all fasting subjects and then serum was separated from whole blood after centrifugation and immediately kept at -20°C until measurement. TGF-β1, MMP-9 and VEGFR-2 were determined by ELISA method using assay kit from R&D Systems, Minneapolis, USA.

Results

The baseline characteristics of the subjects were shown in Table 1.

Table 1. Basic Subject Characteristics (total of 115 patients).

Parameter	Minimum	Maximum	Mean
Age (year)	34	70	51.29
Microalbuminuria (µg/mg K)	1	971	50.46
Fasting Glucose (mg/dL)	66	120	86.59
Triglyceride (mg/dL)	36	560	140.72
Clearance Creatinine	50	215	101.43
Systolic BP	90	200	137.83
Dyastolic BP	60	120	87.74
TGF-β1 (ng/mL)	9.2093	95.7465	35.7808
MMP-9 (ng/mL)	111.5	1548.4	493.5557
VEGFR-2 (ng/mL)	3.3852	16.0481	9.42675

Table 2. Mean level of TGF-β1, MMP-9, and VEGFR-2 in normoalbuminuria normotension, normoalbuminuria hypertension, and microalbuminuria hypertension.

Variable	Normoalbuminuria Normotension (30)	Normoalbuminuria Hypertension (50)	Microalbuminuria Hypertension (30)
TGF-β1	27.63178 ± 12.97246	38.61193 ± 17.09546	38.73939 ± 12.63911
MMP-9	438.1967 ± 156.4268	564.5873 ± 291.2876	418.69 ± 188.3801
VEGFR-2	9.90552 ± 1.85158	9.39561 ± 1.75413	9.00506 ± 1.47173

Table 2 showed that the concentration of TGF-β1 increased from normoalbuminuria normotension to normoalbuminuria hypertension and microalbuminuria hypertension. Concentration of MMP-9 increased from normoalbuminuria normotension to normoalbuminuria hypertension but lower compared to microalbuminuria hypertension. Concentration of VEGFR-2 decreased from normoalbuminuria normotension to normoalbuminuria hypertension and microalbuminuria hypertension.

Table 3. T-test analysis of TGF-β1, MMP-9 and VEGFR-2 in normotension and hypertension.

Variable	Normotension	Hypertension	P
TGF-β1	27.63178 ± 12.97246	38.65692 ± 15.58950	<0.001
MMP-9	438.1967 ± 156.4268	513.0941 ± 267.7983	0.399
VEGFR-2	9.90552 ± 1.85158	9.25777 ± 1.66165	0.078

Table 3 showed that TGF-β1 concentration was higher significantly in hypertensive patients than in normotensive patients ($p<0.001$). No differnece was found between concentrations of MMP-9 and VEGFR-2 among the two groups.

Table 4. T-test analysis of TGF-β1, MMP-9, and VEGFR-2 in normoalbuminuria hypertension and microalbuminuria hypertension.

Variable	Normoalbuminuria Hypertension	Microalbuminuria Hypertension	P
TGF-β1	38.61193 ± 17.09546	38.73939 ± 12.63911	0.541
MMP-9	564.5873 ± 291.2876	418.69 ± 188.3801	0.028
VEGFR-2	9.39561 ± 1.75413	9.00506 ± 1.47173	0.443

Table 4 showed that MMP-9 concentration was higher significantly in normoalbumnuria hypertensive patients than in microalbuminuria hypertensive patients ($p=0.028$). No difference between concentrations of TGF-β1 and VEGFR-2 among two groups was seen.

Table 5. T-test analysis of TGF-β1, MMP-9, and VEGFR-2 in normoalbuminuria normotension and microalbuminuria hypertension.

Variable	Normoalbuminuria Normotension	Microalbuminuria Hypertension	P
TGF-β1	27.63178 ± 12.97246	38.73939 ± 12.63911	< 0.001
MMP-9	438.1967 ± 156.4268	418.6900 ± 188.3801	0.664
VEGFR-2	9.90552 ± 1.85158	9.00506 ± 1.47173	0.042

Table 5 showed that TGF-β1 concentration was higher significantly in microalbuminuria hypertensive patients than in normoalbuminuria normotensive patients ($p<0.001$). No difference was noted between concentrations of MMP-9 and VEGFR-2 among the two groups.

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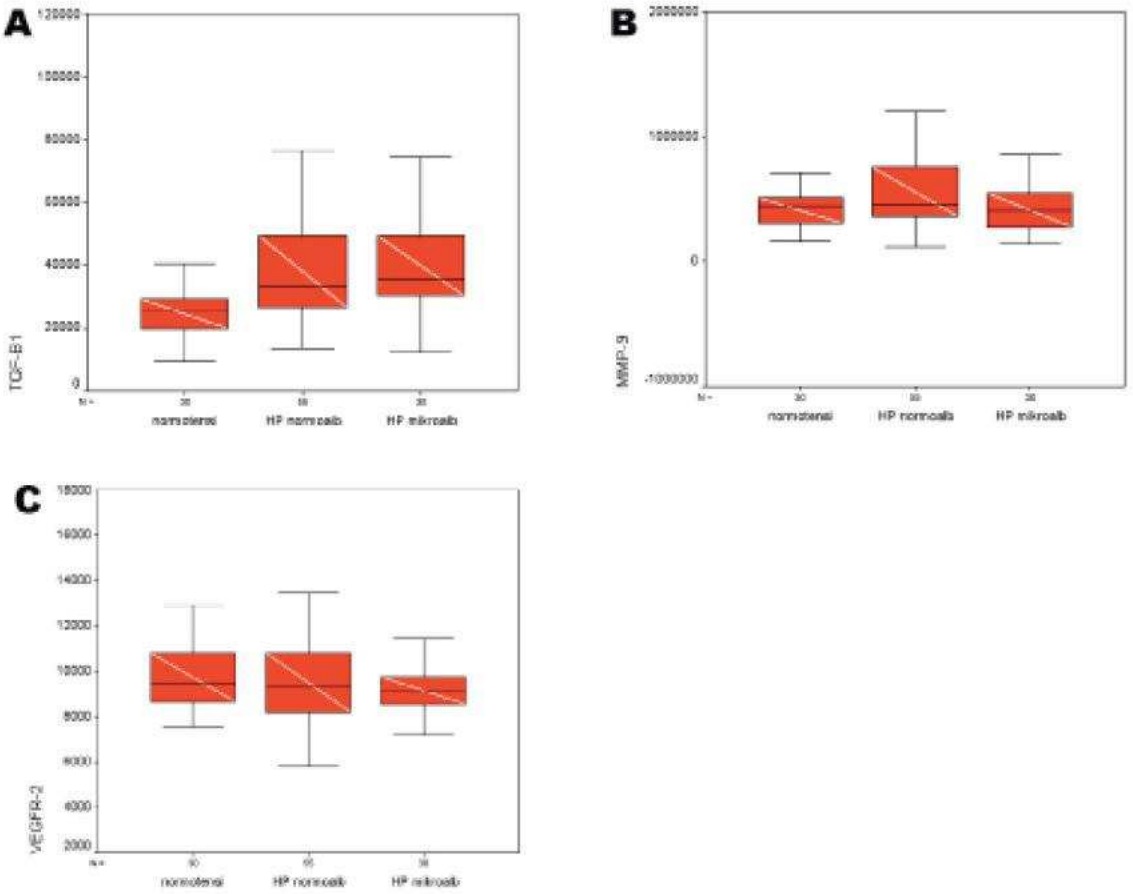

Figure 1. Difference of concentration of TGF-β1 (A), MMP-9 (B), and VEGFR2 (C) in normoalbuminuria normotension, normoalbuminuria hypertension and microalbuminuria hypertension

Discussions

The imbalance process between remodeling and repairing vascular in hypertension was predicted to contribute for the stiffness and thickness of vascular wall that finally would accelerate and yield hypertension complication.

1. Differences of TGF-β1 among normoalbuminuria normotension, normoalbuminuria hypertension and microalbuminuria hypertension

In this study, we found that TGF-β1 concentration increased from normoalbuminuria normotension to normoalbuminuria hypertension and to microalbuminuria hypertension. This could be assumed that remodeling process was increased and occurred in response to a long term changes in hemodynamic condition such as increased blood pressure that contributed to the pathophysiology of vascular disease and circulatory disorders in the future. Some possibilities that may explain this issue are that because in hypertension, increase of blood pressure had positive correlation with the polymorphysm of TGF-β1 gen that impacted to the increasing of TGF-β1 levels. On the other hand, in hypertension cases, the increasing of TGF-β1 expression in vascular wall would induce collagen synthesis in the smooth muscle cell that impacted in accordance to the thickness of vascular wall and finally would worsen the increase of blood pressure.

August et.al (2006) found a relationship between TGF-β1 serum with blood pressure and the relationship between TGF-β1 polimorphysm gen with hypertension subjects. The concentration of TGF-β1 plasma was higher compared to the hypertensive subjects with microalbuminuria and left ventricle hypertrophy (LVH). These results were in accordance with our findings in which we also found the positive correlation between TGF-β1 concentration and systolic and diastolic blood pressure. (6)

2. Differences of MMP-9 among normoalbuminuria normotension, normoalbuminuria hypertension and microalbuminuria hypertension

In this study, we found that MMP-9 concentration was higher in normoalbuminuria hypertension compared to normoalbuminuria normotension, but was lower in microalbuminuria hypertension. We found that MMP-9 concentraion had a positive correlation with systolic and diastolic blood pressure.

Long term increased blood pressure would cause accumulation of Extra Cellular Matrix (ECM) deposits in vascular wall. Increase of ECM would contribute to the progression of LVH and narrowing diameter of the artery luminal. MMP-9 had an important role in the physiology and pathophysiology of remodelling vascular, angiogenesis and arteriosclerosis because MMP-9 had the function to degrade ECM such as collagen type IV and elastin. In this study, we found that MMP-9 concentration was increased from normoalbuminuria normotension to normoalbuminuria hypertension, because with the increase of TGF-β1, MMP-9 also increased to degrade the excessive of ECM deposits. On the other hand, mechanical stretch and shear stress would as well increase MMP-9 synthesis in VSMC and endothelial cell. (7)

Our latest finding revealed that MMP-9 concentration was lower in microalbuminuria hypertension compared to normoalbuminuria normotension and normoalbuminuria hypertension. Some possibilities are that excessive increased of TGF-β1 inhibited MMP mRNA synthesis to yield zymogen (pro-enzyme of MMP). Moreover, TIMP, which functions as MMP inhibitor in controlling ECM metabolism, could inhibit the maturation of MMP from zymogen transformed to be active MMP enzyme. Other possibilities are that microalbuminuria that is a biomarker in predicting vascular disease would give impact to the decrease of MMP-9 expression in hypertensive subjects, whereas, MMP-9 should normally act to compensate the increase of ECM. This would enhance the damage of vascular tone. (7)

3. Differences of VEGFR-2 among normoalbuminuria normotension, normoalbuminuria hypertension and microalbuminuria hypertension

In this study, we found that VEGFR-2 concentration decreased from normoalbuminuria normotension to normoalbuminuria hypertension and to microalbuminuria hypertension. This could be assumed that hypertension could decrease the number of Endothelial Progenitor Cell (EPC). Imanishi at el. (2005) found that hypertension could accelerate EPC senescence through the oxidative stress and telomerase inactivation. Recently an in vitro study showed that oxidized LDL and Ang II induced EPC senescence through oxidative stress. Ang II increased gp91phox expression in EPC, which contributed to oxidative stress by forming peroxynitrite, thiobarbituric acid and 8-epiisoprosyanes, which were positive markers for peroxidized lipid and oxidative stress. Telomerase activation had the function to prolong lifetime and functional activity of endothelial cell. (8)

Conclusions

High concentration of TGF-β1 and lower concentration of MMP-9 in microalbuminuria hypertension might indicate that remodeling process was getting increased. The lower concentration of VEGFR-2 in microalbuminuria hypertension could mean that repairing process was getting decreased.

Acknowledgements:

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