Serum Wnt5a Level is Associated with In-Stent Restenosis in PCI Patients

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Abstract

Objective: To explore the association between serum Wnt5a level and ISR, and to assess the possibility of Wnt5a to be a predictor of ISR.

Methods: 184 bare metal stent (BMS) implanted patients were enrolled in this case-control study. According to coronary angiography results, all patients were divided into 2 groups: in-stent restenosis (ISR) group and non-ISR group. Serum Wnt5a levels were determined using an enzyme-linked immunosorbent assay (ELISA).

Results: Serum Wnt5a levels were higher in ISR group than those in non-ISR group, and were associated with the Gensini score. ISR rate was the highest in the upper tertile of Wnt5a. Multivariate logistic regression indicated that Wnt5a was an independent risk factor of ISR. For diagnosing ISR, the area under ROC curve was 0.817. The cutoff value of Wnt5a diagnosing ISR was 25.83ng/L, with the specificity of 60.68% and sensitivity of 89.55%.

Conclusion: Serum Wnt5a levels is associated with ISR in PCI patients with BMS implantation. Wnt5a is an independent risk factor of ISR, and may act as a biomarker for diagnosing ISR in BMS implanted patients.

Keywords: WNT5A; In-Stent Restenosis; Percutaneous coronary intervention; Bare metal stent.

Introduction

Over the past decades, percutaneous coronary intervention (PCI) with stent implantation is an important treatment for symptomatic coronary artery disease (CAD). However, in-stent restenosis (ISR), which is the most common complication of PCI, plagues cardiologists and patients. Although drug-eluting stents (DES) have been widely used in reducing the incidence of ISR, 5%~10% DES implanted patients are still in the risk of ISR which needs for further revascularization [1]. Therefore, finding the risk factor of ISR is of great significance for predicting and preventing ISR.

ISR occurs in an average of 12 months in DES implanted patients and 6 months in bare-metal stents (BMS) implanted patients post-PCI [2]. The occurrence of ISR is a complex pathophysiological process, and its mechanism is still not quite clear. Vascular injury post-PCI is an important initiating factor, which subsequently induced inflammatory response and vascular reparative process [3]. The vascular response to PCI is associated with cellular functions in many cell types, including endothelial cells and vascular smooth muscle cells (VSMCs) [4].

The Wnt signal transduction cascade plays an important role in many physiological and pathophysiological processes. It’s a main regulator of development of mammals, and affect cell functions in many cell types [5]. Wnt proteins can activate two different pathways. The canonical Wnt signaling depends on β-catenin, while non-canonical Wnt signaling is β-catenin-independent. Wnt5a, which can activate non-canonical Wnt signaling, is a member of Wnt family, and it has recently attracted attention of researchers. It is reported that Wnt5a may be implicated in the regulation of cell proliferation and migration in endothelial cells and VSMCs, which is critical for the occurrence of ISR [6, 7]. However, the clinical significance of Wnt5a in ISR is still not clear.

In this study, we aim to explore the association between serum Wnt5a level and ISR, and to assess the possibility of Wnt5a to be a predictor of ISR in patients with BMS implantation.

Materials and Methods

Study Population

We enrolled 184 patients who had undergone BMS implantation in cardiovascular department of the second affiliated hospital of Xi'an Jiaotong University from June 2016 to June 2017. All patients underwent subsequent Coronary Angiography (CAG) 12 months after PCI, and were divided into 2 groups according to the CAG results: ISR group (n=67) and non-ISR group (n=117). Baseline parameters were collected at admission, including sex, age, Body Mass Index (BMI), Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP) and risk factors of CAD. Current smokers were defined as patients who smoked ≥10 cigarettes per day. Patients whose blood pressure over 140/90 mmHg, or who had been diagnosed with hypertension, were considered current hypertension. The definition of hyperlipidemia was described previously [8]. ISR was defined as diameter stenosis >50% at the stent segment or 5mm segment adjacent to the stent [1]. The exclusion criteria including: (1) dysfunction of liver or kidney; (2) cancer; (3) autoimmune disease; (4) severe infection; (5) diabetes; (6) heart failure; (7) valvular heart disease; (8) cardiomyopathy. The patients provided written informed consent after explanation of the study, and this study was performed under medical ethics.
CAG and Gensini score

All patients had undergone subsequent CAG. CAG and Gensini score assessment was performed as previously described [9].

Blood sample and laboratory test

Blood samples were obtained the day after admission (after 8 hours fasting). The standard methods was performed to determine serum levels of Blood Urea Nitrogen (BUN), Creatinine (Cr), Fasting Blood Glucose (FBG), Total Cholesterol (TC), Triglyceride (TG), High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL). Serum Wnt5a (Cusabio Technology LLC, Wuhan, China) and IL-6 (Protein tech Group, Inc, America) levels were determined by enzyme-linked immunosorbent assay (ELISA).

Statistical analysis

Statistical analyses were performed by using SPSS 18.0 software. Continuous data was expressed as mean±SD, and Student’s t-test was applied to compare means between groups. One-way ANOVA with the LSD post hoc multiple comparisons were applied to compare means among 3 or more groups.

Table 1: Baseline characteristics of patients

<table>
<thead>
<tr>
<th></th>
<th>ISR(n=67)</th>
<th>Non-ISR(n=117)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male %)</td>
<td>47 (70.15%)</td>
<td>73 (62.39%)</td>
<td>0.288</td>
</tr>
<tr>
<td>Age</td>
<td>62.2±10.76</td>
<td>60.5±8.77</td>
<td>0.223</td>
</tr>
<tr>
<td>BMI</td>
<td>21.69±1.85</td>
<td>21.51±2.13</td>
<td>0.566</td>
</tr>
<tr>
<td>SBP</td>
<td>132.79±19.68</td>
<td>131.20±15.60</td>
<td>0.546</td>
</tr>
<tr>
<td>DBP</td>
<td>76.93±11.57</td>
<td>75.65±10.06</td>
<td>0.435</td>
</tr>
<tr>
<td>Gensini score</td>
<td>66.75±12.28</td>
<td>33.11±10.96</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoking</td>
<td>44 (65.67%)</td>
<td>66 (56.41%)</td>
<td>0.218</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>38 (56.72%)</td>
<td>53 (45.30%)</td>
<td>0.136</td>
</tr>
<tr>
<td>Hypertension</td>
<td>38 (56.72%)</td>
<td>52 (44.44%)</td>
<td>0.109</td>
</tr>
<tr>
<td>Culprit vessel</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LM</td>
<td>5 (7.6 %)</td>
<td>7 (5.98%)</td>
<td>0.696</td>
</tr>
<tr>
<td>LAD</td>
<td>55 (82.09%)</td>
<td>89 (76.07%)</td>
<td>0.341</td>
</tr>
<tr>
<td>LCX</td>
<td>35 (52.24%)</td>
<td>47 (40.17%)</td>
<td>0.113</td>
</tr>
<tr>
<td>RCA</td>
<td>49 (73.13%)</td>
<td>86 (73.50%)</td>
<td>0.956</td>
</tr>
<tr>
<td>Laboratory test</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>FBG(mmol/L)</td>
<td>5.22±0.63</td>
<td>5.12±0.59</td>
<td>0.294</td>
</tr>
<tr>
<td>BUN(mmol/L)</td>
<td>5.04±1.23</td>
<td>4.81±1.27</td>
<td>0.233</td>
</tr>
<tr>
<td>Cr(μmol/L)</td>
<td>73.77±16.43</td>
<td>70.80±14.56</td>
<td>0.206</td>
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<tr>
<td>TC(mmol/L)</td>
<td>4.14±0.97</td>
<td>4.18±1.09</td>
<td>0.818</td>
</tr>
<tr>
<td>TG(mmol/L)</td>
<td>1.78±1.17</td>
<td>1.65±1.02</td>
<td>0.411</td>
</tr>
<tr>
<td>HDL(mmol/L)</td>
<td>1.04±0.27</td>
<td>1.10±0.28</td>
<td>0.139</td>
</tr>
<tr>
<td>LDL(mmol/L)</td>
<td>2.61±0.87</td>
<td>2.57±0.80</td>
<td>0.758</td>
</tr>
<tr>
<td>Wnt5a(ng/L)</td>
<td>32.48±7.75</td>
<td>24.63±4.72</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-6(pg/ml)</td>
<td>48.51±3.44</td>
<td>45.60±2.77</td>
<td>&lt;0.001</td>
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BMI: Body Mass Index; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; LM: Left Main Coronary Artery; LAD: Left Anterior Descending Branch; LCX: Left Circumflex Branch; RCA: Right Coronary Artery FBG: Fasting Blood Glucose; BUN: Blood Urea Nitrogen; Cr: Creatinine; TC: Total Cholesterol; TG: Triglyceride; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein

Categorical data was expressed as numbers and percentages, and Chi-square test was used to seek difference between groups. Spearman correlation analysis was used to explore the relationship between Wnt5a and Gensini scores. Univariate and multivariate logistic regression analysis was used to explore the relationship between Wnt5a and ISR. The potential of Wnt5a diagnosing ISR was assessed by ROC curve analysis. P<0.05 was considered statistically different.

Results

There are 67 patients in ISR group and 117 patients in non-ISR group. The clinical characteristics are listed in Table 1. No significant differences were observed between 2 groups regarding gender, sex, BMI, SBP , DBP and risk factors of CAD. CAG results showed that there are no significant differences regarding culprit vessel between 2 groups. Gensini score is significantly higher in ISR group (66.75±12.28) than that in non-ISR group (33.11±10.96). As for laboratory test, FBG, BUN, Cr, TC, TG, HDL, LDL are all at similar levels in 2 groups. Serum Wnt5a and IL-6 levels are significantly higher in ISR group than those in non-ISR group. Spearman correlation analysis showed that Wnt5a is associated with Gensisn score (r=0.580, P<0.001, Figure 1).

BMI: Body Mass Index; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; LM: Left Main Coronary Artery; LAD: Left Anterior Descending Branch; LCX: Left Circumflex Branch; RCA: Right Coronary Artery FBG: Fasting Blood Glucose; BUN: Blood Urea Nitrogen; Cr: Creatinine; TC: Total Cholesterol; TG: Triglyceride; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein
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Figure 1: Correlation between Wnt5a and Gensini scores
Spearman correlation analysis showed that serum Wnt5a levels were associated with Gensini scores (r=0.580, P<0.001).

Figure 2: ISR rate in different tertile of serum Wnt5a levels
ISR rate was the highest in tertile 3 (38/61) than those in tertile 2 (25/62) and tertile 1 (4/61).

Figure 3: SROC curve for Wnt5a predicting ISR

Discussion

ISR is a major complication of PCI, which adversely affects patients’ life quality and prognosis. Exploring the risk factors of ISR is of great clinical significance for predicting and preventing the occurrence of ISR. In the current study, we found that serum Wnt5a level is associated with Gensini score in PCI patients with BMS implantation, which indicated that Wnt5a may play a role in the development of coronary artery stenosis. It is reported that Wnt5a was involved in various physiological processes such as proliferation, migration, inflammation and lipid metabolism, each of which is essential for the development of atherosclerosis [10]. Moreover, we report for the first time that serum Wnt5a is an independent risk factor of ISR, and there is possibility of Wnt5a to be a predictor of ISR in BMS implanted patients.

The mechanism of ISR is not fully elucidated. Kumatsu et al., investigated 11 stented coronary arteries in 11 patients. Histological findings indicated that inflammation and neointima proliferation is critical for the formation of ISR [11]. After PCI, the presence of stents can induce acute inflammation. Adhesion of acute inflammatory cells, including neutrophils and monocytes, is a character of early vascular response. Over weeks, the chronic...

Table 2: Univariate and multivariate logistic regression of Wnt5a and ISR risk

<table>
<thead>
<tr>
<th>Model</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>Univariate</td>
<td>1.287 (1.179,1.404)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adjusted model 1</td>
<td>1.287 (1.177,1.408)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adjusted model 2</td>
<td>1.284 (1.174,1.405)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adjusted model 3</td>
<td>1.256 (1.129,1.397)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

OR : odds ratio
Model 1: Adjusted for sex, age and BMI
Model 2: Adjusted for model 1 and risk factors of CAD
Model 3: Adjusted for model 2 and TC, TG, LDL, HDL and IL-6
inflammatory cells, including macrophages, replace acute inflammatory cells [4, 12]. Wnt5a is an important regulator of inflammation. Christman et al. reported that Wnt5a expressed in advanced atherosclerosis plaques, which is characterized by macrophage aggregation [13]. Recombinant Wnt5a could induce inflammatory cytokines production of macrophages via TLR4 dependent pathways, including IL-6 [14]. Our data showed that serum IL-6 levels, an important inflammatory cytokine, is higher in ISR group than those in non-ISR group. Multivariate logistic regression suggested that Wnt5a is independently associated with the presence of ISR, even adjusted for IL-6. This result indicates that Wnt5a may affect ISR by some other mechanism except for inflammation.

Neointima tissue proliferation is an important mechanism of ISR. This pathological process involves functional changes in various vascular wall cells. As the inner layer of arteries, endothelial cells play important roles in ISR process. Vascular endothelium integrity is an important prerequisite for protection of blood vessel [15]. However, PCI operation, including barotraumas and stimulation of stent, could damage vascular endothelium, which could induce the proliferation, migration and apoptosis of endothelial cells [16]. Wnt5a is reported to be a regulator of endothelial cells. Maskauchan et al. found that Wnt5a is expressed in human endothelial cells, and it could induce the proliferation and survival of endothelial cells. Endothelial cells migration was inhibited by reduced Wnt5a expression [17]. Kim et al. found that Wnt5a induced release of inflammatory cytokines, such as IL-6 and IL-8, via Wnt/Ca²⁺ signaling pathway. This result suggests that Wnt5a may act as an inflammatory mediator in endothelial cells [18].

Endothelial cell layer can inhibits VSMCs proliferation and intimal hyperplasia under normal circumstance, and VSMCs express high levels of contractile proteins. After vascular injury, phenotypic transformation may occur in VSMCs, which is characterized by increased level of proliferation, migration and extracellular matrix synthesis [19]. It is reported that the stable neointima contains 20% VSMCs [20], suggests that VSMCs play an important role in ISR. Pandey et al. found that Wnt5a mRNA was expressed in human VSMCs, as well as its receptors, such as Fzd1, Fzd2 and Fzd5 [21]. This result suggests that Wnt5a can be implicated in ISR via regulating VSMCs functions. Qin et al. treated VSMC with siRNA of Wnt5a, and found that total and free cholesterol content in VSMC exposed to oxLDL increased significantly, whereas recombinant Wnt5a treatment had the opposite effect [22]. This result suggests that Wnt5a could regulate cholesterol accumulation in VSMC. DiRenzo et al. found that application of recombinant Wnt5a, Wnt12b, Wnt4 or Wnt9a stimulated VSMC proliferation via β-catenin dependent pathways [7]. However, it seems controversial that recombinant Wnt5a or over expression of Wnt5a cannot increase the proliferation of VSMC in another study [23]. Further study is needed to clarify Wnt5a functions in VSMC and underlying mechanisms.

Some limitations need to be noticed. First, this was a single-center study with a relatively small study population. It is necessary to confirm our conclusion in a large sample size study in the future. Second, as a case-control study, we cannot elucidate a causal relationship. Prospective and experimental studies are needed to fully elucidate the relationship between serum Wnt5a levels and ISR and its underlying mechanisms. Third, we did not measure serum Wnt5a levels over time. The dynamic changes in Wnt5a levels may help clarify the role of Wnt5a in the prognosis of ISR.

In conclusion, our data suggests that serum Wnt5a levels are associated with ISR in PCI patients with BMS implantation. Wnt5a is an independent risk factor of ISR, and it may act as a predict biomarker of ISR in BMS implanted patients. Our findings provide new insights into the clinical significance of the Wnt signaling pathway. Further study is needed to elucidate the relationship between Wnt5a and ISR and its underlying mechanisms.

Acknowledgement

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Declarations

No conflict of interest.

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