

# Association of Resistin & Interleukin 6 in Ischemic Heart Disease and Diabetic Patients

Faisal Yaseen<sup>1</sup>

## ABSTRACT

**Objectives:** To measure the serum levels of resistin and interleukin 6 levels in single, two vessel and three vessel disease on angiography in ischemic heart disease patients with diabetes and without diabetes. To determine an association between the biomarkers(resistin, IL-6 levels) and cardiovascular disease.

**Methods:** A comparative cross sectional study was designed in which samples of 147 subjects were selected. Age was in range of 40 to 70 years. Subjects were divided into two groups of 38 diabetics with complication of ischemic heart disease and 38 non diabetic with ischemic heart disease. Plasma glucose was measured using the Glucose oxidase method. Resistin and interleukin-6 were determined by ELISA. Extent of vessel disease was determined by standard angiography method.

**Results:** The total subjects included in the study were 76. Angiographic findings of 76 patients with IHD revealed that, 19 (25%) had single vessel disease (SVD), 16 (21.05%) had two vessels disease (2VD) and 41 (53.95%) had three vessels disease (3VD). Group of 38 non diabetic patients with IHD, 8 (21.1%) had SVD, 7 (18.4%) had 2VD and 23 (60.5%) had 3VD. Among 38 patients of IHD with DM, 11 (28.9%) had SVD, 9 (23.7%) had 2VD and 18 (47.4%) had 3 VD. Positive correlations of Interleukin-6 and resistin in single vessel disease group ( $r=0.190$ ,  $p=0.112$ ) was found, but higher positive correlation in two vessels disease group ( $r=0.240$ ,  $p=0.323$ ) and the highest positive correlation of interleukin-6 and resistin was found in three vessels disease group ( $r=0.324$ ,  $p=0.221$ ).

**Conclusion:** The study showed that the resistin and interleukin plasma concentrations were significantly increased in 3VD which shows the role of these cytokines in ischemic heart disease.

**KEY WORDS:** *Resistin, Interleukin 6, Ischemic Heart Disease.*

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## INTRODUCTION

Cardiovascular disease is one of the leading cause of death worldwide. Some important risk factors of cardiovascular diseases are age, sedentary life style, hypertension, hypercholesterolemia, smoking, insulin resistance, diabetes mellitus and the genetic tendency.<sup>1</sup> The major cause of coronary artery disease is atherosclerosis that leads to the imbalance in the supply and demand of oxygen for the myocardium.<sup>2</sup>

Atherosclerosis, is a complex disease that is characterized by cholesterol deposition in sub endothelial space along with monocyte infiltration and foam cell deposition.<sup>3,4</sup> According to a report by the WHO, by 2010, cardiovascular disease will be the leading of cause of death in developing countries.<sup>5</sup> By 2020, Pakistan will be ranked 4<sup>th</sup> most heavily populated country in terms of diabetic patients and every 3<sup>rd</sup> person above the age of 45 years will be hypertensive<sup>6</sup>. Due to the lack of recreational activities and diet rich in salts and fats Pakistani women are at a higher risk of cardiovascular disease.<sup>7</sup>

Obesity and atherosclerosis are now viewed as the inflammatory states and biomarkers or hormones are now getting more attraction as metabolic signals for the atherosclerotic cardiovascular disease (CVD).<sup>8</sup> Resistin is amongst one of adipocytokine belonging to the family of cysteine-rich secretory proteins called resistin-like molecules<sup>9,10</sup> or FIZZ (found in inflammatory zones) proteins.<sup>11</sup> In rodents model, resistin is almost seen coming exclusively from fat tissue, and is found elevated in the adipose expression and in the models of insulin resistance and obesity.<sup>12,13</sup> Hyper-resistinemia known to impair the glucose tolerance<sup>14</sup> and in rodents induces the hepatic insulin resistance,<sup>15</sup> whereas mice who had deficient levels of resistin are protected from obesity-associated insulin resistance.<sup>16</sup> In humans, resistin is expressed primarily in inflammatory cells as contrast to rodents.<sup>17-19</sup> Resistin expression in human monocytes was markedly increased by treatment with endotoxin and pro inflammatory cytokines<sup>18</sup> and some other cytokines had an association between it and circulating interleukin 6 (IL-6) levels as major mediators of inflammation<sup>14</sup>, and these are strongly associated with obesity and cardiovascular

disease.<sup>15,16</sup>

Beside this, measurement of the inflammatory marker IL-6 has been postulated as a method of determining increased risk of cardiovascular disease in apparently healthy individuals.<sup>19</sup> There is also increasing evidence that systemic inflammation and insulin resistance constitute interrelated events that contribute to atherosclerosis. The study was designed to correlate levels of these cytokines to the extent of vessel disease involvement as determined by angiography.

## METHODOLOGY

The study included 147 subjects, with age range of 40 to 70 years. They were divided into two groups which include 38 diabetic patients with IHD and 38 non diabetic with IHD. Convenient sampling was done and samples were collected from University Hospitals, Medical and Diabetic Centre and Heart Centre. The consent was obtained from each subject and study was approved by Ethical committee. Patients with cardiac complications other than IHD, having endocrine disorders or on hormone replacement therapy or patients on Glitazone therapy were excluded from the study.

At each visit, height and weight was measured with the participant in light clothing. BMI was estimated as weight (in kilograms) divided by height (in meters) squared. BMI is classified as: Underweight <18.5, Normal Range= 18.5-22.9, Over weight=23-24.9, Obese >25. Waist and hip circumferences was measured in duplicate using a metal tape. On the basis of waist / hip ratio, subjects are classified as obese if the ratio is greater than: 0.9 in males, 0.8 in females.<sup>20</sup>

Blood samples were drawn after 12 hours of fasting, and blood glucose level was determined within 1 hour of sample collection by glucose oxidase method.<sup>21</sup> Samples were stored at -70 °C till further analysis. Resistin was determined by ELISA (SPI Bio France).<sup>22</sup> Interleukin-6 was measured by ELISA (DRG diagnostics kit).<sup>23</sup> Angiography was performed on TOSHIBA Infinix 2000 by a consultant cardiologist on the basis of history, signs and symptoms, cardiac enzymes levels and ECG findings.

Data analysis was done by using SPSS (Statistical program for social sciences) version-17.0. Qualitative variables like gender were

presented by frequencies and percentages. Chi-square test was applied to compare gender among four groups of the study. All continuous response variables like age, BMI, waist hip ratio, fasting blood glucose, interleukin-6 and resistin levels were presented by Mean  $\pm$  SD. Analysis of variance was performed to compare two study groups and post-Hoc Scheffe's test was applied to compare pair-wise groups. Test of linear correlation (r) was applied to assess relationship of blood parameters among the two groups. Scatter plots were also made to show the coefficient of correlation and prediction regression equations. Statistical significance was set at 5% level of significance and  $p \leq 0.05$  was considered statistically significant result.

### RESULTS

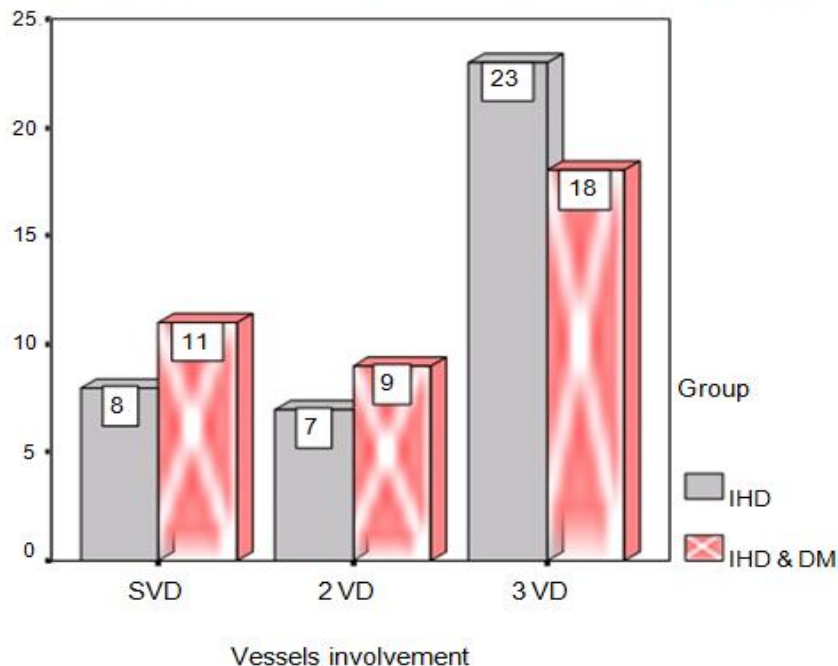
The total subjects included in the study were 76. Angiographic findings of 76 patients with IHD showed that, 19 (25%) had single vessel disease (SVD), 16 (21.05%) had two vessels disease (2VD) and 41 (53.95%) had three vessels disease (3VD). Group of 38 non diabetic patients with IHD, 8 (21.1%) had SVD, 7

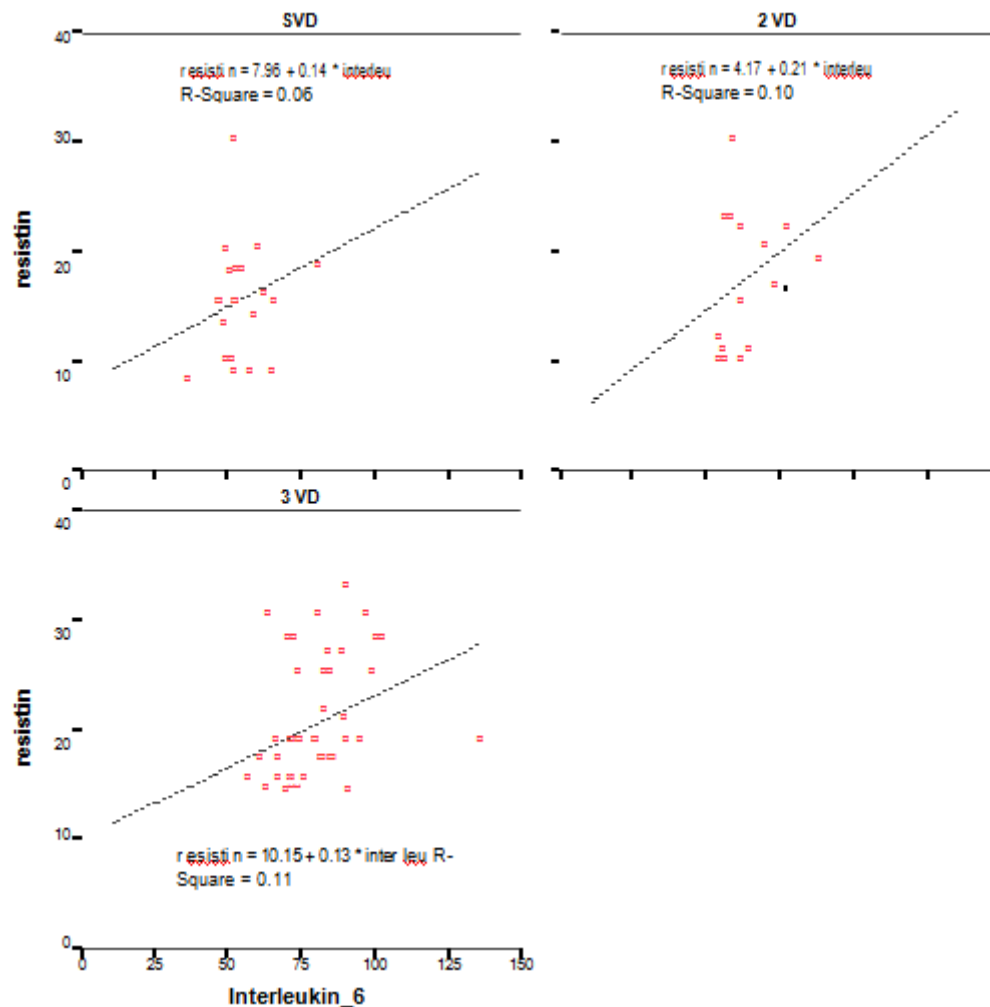
(18.4%) had 2VD and 23 (60.5%) had 3VD. Among 38 patients of IHD with DM, 11 (28.9%) had SVD, 9 (23.7%) had 2VD and 18 (47.4%) had 3 VD. Non-significant difference was found in the concentrations of the two cytokines amongst SVD, 2VD and 3VD in diabetic and non-diabetic groups with IHD. Data reveals severity of IHD in two groups (Chi-square = 1.33,  $p=0.513$ ) as shown in Figure 1.

Mean comparison of interleukin-6 and resistin levels according to severity of ischemic heart disease groups on the basis of angiographic findings were done. The mean Interleukin-6 of SVD group was  $55.23 \pm 9.38$  which was statistically similar with the mean interleukin-6 of 2VD group ( $p=0.156$ ) but the mean Interleukin-6 of 3VD group was statistically significant than the mean of SVD and 2VD group ( $p<0.001$ ) as shown in Table 1. The mean resistin of SVD group was  $15.82 \pm 5.57$  which was statistically similar with the mean Interleukin-6 of 2VD groups ( $p=0.647$ ) but the mean Interleukin-6 of 3VD group was statistically significantly than the mean of 2VD group ( $p=0.012$ ) as shown in Table 1.

**Figure 1: Distribution of Ischemic Heart Disease According to Severity**

Insignificant difference of severity of IHD in two groups (Chi-square = 1.33,  $p=0.513$ )



**Figure 2: Correlation between Resistin & Interleukin-6 in Patients with Vessels Involvement**

Positive correlations of Interleukin-6 and resistin in single vessel disease group ( $r=0.190$ ,  $p=0.112$ ) was found, but higher positive correlation in two vessels disease group ( $r=0.240$ ,  $p=0.323$ ) and the highest positive correlation of interleukin-6 and resistin was found in three vessels disease group ( $r=0.324$ ,  $p=0.221$ ). However, all these correlations were statistically non-significant at 95% level of significance Figure 2.

## DISCUSSION

The study was designed to explore the variation in the levels of resistin and IL-6 in ischemic heart disease patients with and without diabetes and to correlate them with the extent of vessel disease as determined by

angiography. The study shows increased plasma concentration of cytokines as the number of vessels affected increased showing association of these cytokines with them. However non-significant difference was found in diabetic and non-diabetic patients with IHD.

In a study done by Reilly et al. in 2005 it was found out that resistin was not only associated with coronary artery calcification (CAC) a measure of atherosclerosis but also positively correlated with IL-6 which was linked to the insulin resistance, obesity and atherosclerotic CVD.<sup>24</sup> Recent studies have highlighted the importance of inflammation in linking obesity and the occurrence of the metabolic syndrome, suggesting that the metabolic syndrome is a low-grade systemic inflammatory condition.<sup>25</sup> Although

this emerging concept of inflammation has been recognized in the context of increasing adiposity, its molecular basis and physiological significance in terms of the pathogenesis of type 2 diabetes mellitus is yet to be fully elucidated. Thus adipose tissue may act as a site for mediation of this chronic inflammation through the action of known pro-inflammatory adipocytokines such as leptin, TNF- $\alpha$ , IL-6 and, most recently proposed, resistin.<sup>26</sup>

IL-6 in older adults has been examined also in some studies. Cesari et al. 2003<sup>27</sup> found IL-6 to be a better predictor of incident coronary disease than CRP. Its relationship to incident stroke was particularly strong (RR=3.7; 95% CI: 1.67-8.21). Harris et al. 1999<sup>28</sup> also found IL-6 to be a stronger predictor than CRP for predicting cardiovascular mortality. Resistin levels seen elevated in patients presenting with unstable angina, non ST segment elevation myocardial infarction and ST segment elevation myocardial infarction in a study done by Lubos et al. 2006 and were thought to play a role as a diagnostic marker.<sup>29</sup>

Our findings concerning the higher plasma levels IL-6 plasma levels and resistin in diabetic subjects with complications of ischemic heart disease (IHD) in comparison with diabetics without IHD may confirm this topics. The study done by Tuttolomondo et al. 2010 also showed high resistin and IL6 levels in the diabetics with and without complications of diabetic foot, which also gives an idea of an association of atherosclerosis and micro/ macrovascular complications with higher circulating levels of aforementioned

adipokines.<sup>30</sup> The results in the study done by Cho et al. 2011 also indicate that resistin aggravates atherosclerosis by stimulating monocytes, endothelial cells, and vascular smooth muscle cells to induce vascular inflammation. These findings provide the first insight on the causal relationship between resistin and atherosclerosis.<sup>31</sup> Despite all this still there is some debate as to whether inflammatory markers are signs of an active on-going disease process or causal determinants for the development of disease. The issue is important because of the implications for developing and targeting therapies. The importance of inflammatory processes in the progression of atherosclerotic disease and thus still remains the high interest research topic.

## CONCLUSION

The study points out the important role of resistin and interleukin 6 in ischemic heart disease the extent and number of vessels involved. Moreover an interesting finding is the significant positive correlation in the plasma levels of these cytokines with three vessel disease. A large study based on similar population needs to be carried out to establish these cytokines as marker or predictor of cardiovascular disease.

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