



# ATHEROSCLEROSIS; ASSOCIATION OF ADIPONECTIN/LEPTIN RATIO TO INTIMA MEDIA THICKNESS IN LOCAL POPULATION

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**ABSTRACT... Objectives:** To compare the adiponectin/leptin ratio and intima media thickness between normal and atherosclerotic individuals. To find out the association of A/L ratio with IMT in atherosclerotic patients. **Study Design:** Comparative cross sectional study. **Place and Duration of Study:** This study was conducted at department of Physiology, BMSI in collaboration with Radiology department, Jinnah Postgraduate Medical Centre Karachi, from 16th December 2014 to 15th December 2015. **Methodology:** 160 subjects having Doppler ultrasound of neck were selected on the basis of pre-determined criteria. They were grouped in cases i.e. atherosclerotic group (80 subjects) and control i.e. healthy group (80 subjects). Age > 20 years, both genders, patients for ultrasound Doppler for neck region irrespective of their provisional diagnosis, patients suffering from co-morbid conditions like diabetes, hypertension, chronic kidney disease, chronic liver disease, asthma and COPD and Healthy individuals (Controls) were included in this study. Patients who fail to give consent and patients whose ultrasound findings were not up to the mark required for diagnosis were excluded from this study. **Results:** Out of 160 patients in this study in control Male: Female ratio was 1:1.1 while in cases, Male: Female ratio = 1:1. Majority (53.1%) of cases had age >50 years {41(51.25%) in controls and 44(55%) in cases}. Mean ages in two groups (controls and cases) were 49.39+12.30 years and 53.31+10.09 years respectively ( $p = 0.029$ ). 44 (22.5%) patients were smoker and 116 (72.5%) were non-smoker. In control group, 13 (16.3%) were smokers whereas in cases 31 (38.8%) were smokers ( $p = 0.002$ ). Mean triglyceride levels were statistically high in atherosclerotic subjects as compared to healthy individuals (138.31+62.65 mg/dl and 125.73+46.17 mg/dl respectively;  $p=0.024$ ). Mean IMT of both right and left carotid arteries examined via carotid Doppler ultrasound were statistically high in atherosclerotic individuals as compared to healthy subjects {(Right carotid; 0.83+0.23 mm and 0.63+0.16 mm respectively;  $p=0.022$ ) (Left carotid; 0.85+0.25 mm and 0.64+0.16 mm respectively;  $p=0.004$ )}. Mean Leptin levels were statistically high in atherosclerotic individuals as compared to healthy subjects (332.32+555.31 ng/ml and 254.55+224.66 ng/ml respectively;  $p=0.001$ ). However, the difference in Adiponectin levels was insignificant (7.93+4.80  $\mu\text{g/ml}$  and 9.45+4.73  $\mu\text{g/ml}$  respectively;  $p=0.343$ ). **Conclusion:** We conclude that plasma levels of Adiponectin and Leptin are significantly higher in atherosclerotic than normal individuals.

**Key words:** Adiponectin, Leptin, Atherosclerosis, Adiponectin / Leptin ratio.

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## INTRODUCTION

Atherosclerosis, a chronic inflammatory disease process of complex etiology, consist of gradual progress over the period of time start from the development of endothelial injury to the fibrous cap and thrombus formation in the advance plaque.<sup>1</sup> The exact cause of atherosclerosis is not known. Major risk factors include unhealthy blood cholesterol levels, high blood pressure, smoking, obesity and diabetes etc.<sup>2</sup> Emerging risk factors include low C reactive proteins (CRP) levels and

sleep apnea etc. Key process in the development of atherosclerosis is low density lipoprotein (LDL) oxidation and accumulation in vascular cells, promoting foam cell formation as well as increased secretion of mediators of inflammation. The inflammatory state, in turn can induce oxidative stress by enhancing the production of reactive oxygen species in the vascular wall.<sup>3</sup>

Leptin is a 167-amino acid circulating protein mainly produced by white adipose tissue and

its production is regulated by energy level, food intake, several hormones, and various inflammatory mediators. It also has distinct cytokine properties, such as the stimulation of inflammation and modulation of the immune system.<sup>4,5</sup> Hyperleptinemia is associated with atherosclerosis, hypertension and metabolic syndrome. Increased serum concentrations of leptin are also associated with an increased risk of myocardial infarction and stroke and cardiovascular risk factors.<sup>4</sup>

Adiponectin is a 244-AA protein. The role of adiponectin is multifarious; it has insulin sensitizing, anti-inflammatory, and anti-atherogenic properties. It has been displayed that Adiponectin hampers different mechanisms leading to atherogenesis.<sup>6,7</sup> Serum Adiponectin has been affiliated with cardiovascular events and mortality associated with it inclusive of peripheral artery disease. Deficiency of Adiponectin leads to increased atherosclerosis.<sup>7,8</sup>

Carotid Intima media thickness (IMT) is seemed as the distance between the lumen intima and the media-adventitia interfaces seen on ultrasound images of the carotid artery wall.<sup>9</sup> The common carotid artery (CCA) is often used as a site to measure (IMT). However, there are different approaches to measuring (IMT). One approach recommends using the (CCA) lower than the carotid bulb. Another extends the site of measurement into the carotid bulb, the dilation seen before the origins of the internal and external carotid arteries.<sup>9</sup> Carotid intima-media thickness (IMT) can provide relationship between risk factors and carotid atherosclerosis. IMT is augmented in people with cardiovascular risk factors and a solo predictor of future stroke and myocardial infarction.<sup>10</sup> Hence, it is believed to be an early marker of atherosclerosis.<sup>11,12</sup>

Rationale of the study was serum adiponectin and leptin levels are predictors of the presence and degree of coronary atherosclerosis. In Pakistan to the best of our knowledge work has not been conducted. Our study provided introspection on the relationship between adiponectin, leptin levels and the A/L ratio with IMT in patients referred from

the OPD and in hospital patients to the radiology department for ultrasound IMT. It will also help in understanding the clinical significance of plasma adiponectin and leptin levels which can be helpful in preventing the development of atherosclerotic vascular diseases.

## MATERIAL & METHODS

This study was conducted at department of Physiology, BMSI in collaboration with Radiology department, Jinnah Postgraduate Medical Centre Karachi, from 16th December 2014 to 15th December 2015.

160 subjects having Doppler ultrasound of neck were selected on the basis of pre-determined criteria. They were grouped in cases i.e. atherosclerotic group (80 subjects) and control i.e. healthy group (80 subjects). Age > 20 years, both genders, patients for ultrasound Doppler for neck region irrespective of their provisional diagnosis, patients suffering from co-morbid conditions like diabetes, hypertension, chronic kidney disease, chronic liver disease, asthma and COPD and Healthy individuals (Controls) were included in this study. Patients who fail to give consent and patients whose ultrasound findings were not up to the mark required for diagnosis were excluded from this study. Blood sample for serum levels of adiponectin and leptin was taken for analysis. The blood samples were taken after 12 hours overnight fasting. Serum levels of adiponectin and leptin were measured on ELISA (EA-2500 and KAP 2281, DRG International, Inc.) on a Dynex analyzer. Ultrasound was used for carotid intimal thickening, using a B-mode ultrasound scanner (Siemens Sonoline Sienna Model No. AY-15CUI) and a 7.5 MHz linear probe with subjects in the supine position.

## RESULTS

Out of 160 patients in this study in control Male: Female ratio was 1:1.1 while in cases, Male: Female ratio = 1:1. Majority (53.1%) of cases had age >50 years {41(51.25%) in controls and 44(55%) in cases} (Table-I). Mean ages in two groups (controls and cases) were 49.39±12.30 years and 53.31±10.09 years respectively (p = 0.029) (Table-II).

	Cases n (%)	Control n (%)	P-value*
<b>Gender</b>	M: 40 (50) F: 40 (50)	M: 36 (45) F: 44 (55)	
Smoking	31 (38.8)	13 (16.2)	0.002
<b>Co-morbidities</b>			
Hypertension	32 (40)	04 (8.8)	0.001
Diabetes	28 (35)	07 (5)	
Stroke	04 (5)	00 (0)	
Chronic kidney disease	04 (5)	00 (0)	
None	12 (15)	69 (86.2)	

**Table-I. Patient's characteristics**  
\*Chi square test

44 (22.5%) patients were smoker and 116 (72.5%) were non-smoker. In control group, 13 (16.3%) were smokers whereas in cases 31 (38.8%) were smokers ( $p = 0.002$ ) (Table-I).

Majority of the patients (24.4%) in this study had hypertension as co-morbidity. The second commonly observed co-morbidity was diabetes mellitus (20% patients) ( $p = 0.001$ ) (Table-I).

Mean of weight, height and BMI. The mean BMI was statistically high in atherosclerotic individuals as compared to healthy individuals ( $27.52 \pm 6.24$  kg/m<sup>2</sup> and  $25.79 \pm 4.13$  kg/m<sup>2</sup> respectively;  $p=0.001$ ) (Table-II). Mean triglyceride levels were statistically high in atherosclerotic subjects as compared to healthy individuals ( $138.31 \pm 62.65$  mg/dl and  $125.73 \pm 46.17$  mg/dl respectively;  $p=0.024$ ). Random blood sugar was statistically high in cases as compared to control ( $197.70 \pm 82.91$  mg/dl vs.  $145.75 \pm 21.49$  mg/dl respectively;  $p=0.001$ ). Mean IMT of both right and left carotid arteries examined via carotid Doppler ultrasound were statistically high in atherosclerotic individuals as compared to healthy subjects {(Right carotid;  $0.83 \pm 0.23$  mm and  $0.63 \pm 0.16$  mm respectively;  $p=0.022$ ) (Left carotid;  $0.85 \pm 0.25$  mm and  $0.64 \pm 0.16$  mm respectively;  $p=0.004$ )} (Table-II).

Mean Leptin levels were statistically high in atherosclerotic individuals as compared to healthy subjects ( $332.32 \pm 555.31$  ng/ml and  $254.55 \pm 224.66$  ng/ml respectively;  $p=0.001$ ).

However, the difference in Adiponectin levels was insignificant ( $7.93 \pm 4.80$   $\mu$ g/ml and  $9.45 \pm 4.73$   $\mu$ g/ml respectively;  $p=0.343$ ). Mean Adiponectin-Leptin ratio in control and cases was  $0.05 \pm 0.04$  and  $43.82 \pm 72.73$  respectively ( $p=0.001$ ) (Table-II).

	Cases (Mean $\pm$ SD)	Control (Mean $\pm$ SD)	P-value*
Age (kg)	53.31 $\pm$ 10.09	49.39 $\pm$ 12.30	0.029
<b>Anthropometric measurements</b>			
Weight (kg)	71.43 $\pm$ 15.31	65.20 $\pm$ 12.38	0.052
Height (cm)	161.45 $\pm$ 9.47	158.70 $\pm$ 8.91	0.675
BMI (kg/m <sup>2</sup> )	27.52 $\pm$ 6.24	25.79 $\pm$ 4.13	0.001
<b>Lipid profile</b>			
Triglycerides (mg/dl)	138.31 $\pm$ 62.65	125.73 $\pm$ 46.17	0.024
Cholesterol (mg/dl)	187.65 $\pm$ 49.37	175.25 $\pm$ 40.74	0.185
HDL (mg/dl)	43.06 $\pm$ 9.45	46.54 $\pm$ 7.78	0.130
LDL (mg/dl)	111.51 $\pm$ 39.45	110.09 $\pm$ 35.10	0.664
<b>Blood sugar</b>			
FBS (mg/dl)	146.11 $\pm$ 40.97	131.73 $\pm$ 51.04	0.198
RBS (mg/dl)	197.70 $\pm$ 82.91	145.75 $\pm$ 21.49	0.001
<b>IMT</b>			
Right carotid artery (mm)	0.83 $\pm$ 0.23	0.63 $\pm$ 0.16	0.022
Left carotid artery (mm)	0.85 $\pm$ 0.25	0.64 $\pm$ 0.16	0.004
Leptin levels (ng/ml)	332.32 $\pm$ 555.31	254.55 $\pm$ 224.66	0.001
Adiponectin levels ( $\mu$ g/ml)	7.93 $\pm$ 4.80	9.45 $\pm$ 4.73	0.343
A/L Ratio	43.82 $\pm$ 72.73	0.05 $\pm$ 0.04	0.001

**Table-II. Patient's parameters**

## DISCUSSION

The contemporary mechanism of atherosclerogenesis is inflammatory.<sup>13</sup> The adipokines (leptin, adiponectin and inflammatory cytokines) secreted by adipose tissues are primarily responsible for maintaining arterial tone. Any alterations in adipokines especially adiponectin and leptin lead to atherosclerosis formation.<sup>14</sup> It has been suggested that hyperleptinemia is associated with hypertension, atherosclerosis and metabolic syndrome.<sup>4</sup> Initially, it was suggested in experimental studies that the plasma adiponectin has anti-atherosclerotic and

anti-inflammatory effects. Later, its affiliation with atherosclerosis and cardiovascular risks has been established.<sup>15</sup> Low plasma levels of adiponectin are associated with atherosclerosis.<sup>7,16</sup> Therefore, to elucidate the role of adiponectin-leptin ratio and carotid IMT between normal and atherosclerotic patients, this study was performed.

The male to female ratio was 1:1.1. In control, 36 (45%) were males and 44 (55%) were female, (Male: Female ratio was 1:1.1) while in cases, 40 (50%) were males and females (Male: Female ratio = 1:1). However the male to female ratio given by Vasilescu<sup>17</sup> et al. in 2010 is 1.2:1 which is quite different from present study. The age ranged from 20 to 86 years in both groups with overall mean age was  $51.35 \pm 11.39$  years whereas mean ages in two groups (controls and cases) were  $49.39 \pm 12.30$  years and  $53.31 \pm 10.09$  years respectively, difference between two means was statistically significant ( $p$ -value = 0.029). Majority (53.1%) of cases had age >50 years {41(51.25%) in controls and 44(55%) in cases} which is comparable to other study conducted by Gardener et al. in 2012<sup>18</sup> and reported mean age at baseline was  $66 \pm 9$  years.

Study of Kotani et al. 2005<sup>20</sup> reported smoking status was also a risk for increased carotid intima-media thickness levels, while in our study, 44 (22.5%) patients were smoker and 116 (72.5%) were non-smoker. In control group, 13 (16.3%) were smokers whereas in cases 31 (38.8%) were smokers; the difference between two groups was statistically significant ( $p=0.002$ ).

More recently, the evaluation of the leptin: adiponectin ratio (L:A) has been suggested as an atherosclerotic index in patients with type 2 diabetes and a useful parameter to assess insulin resistance in patients with and without diabetes.<sup>20</sup> In our study second commonly observed co-morbidity was diabetes mellitus (20% patients). The difference between control and cases in term of co-morbidities was statistically significant ( $p = 0.001$ ). However study of Gardener<sup>18</sup> et al. reported that the relationship between adiponectin and IMT was stronger among those with diabetes. In the study of Maher et al.<sup>21</sup>, increased vascular

stiffness associated with significantly greater with body mass index (BMI). In our study mean BMI was statistically high in atherosclerotic individuals as compared to healthy individuals ( $27.52 \pm 6.24$  kg/m<sup>2</sup> and  $25.79 \pm 4.13$  kg/m<sup>2</sup> respectively;  $p \leq 0.001$ ).

In this cross sectional comparative study, 160 individuals (80 Atherosclerosis i.e. cases and 80 age, gender matched controls) were selected. Their ultrasound of the neck was done to determine the IMT of both right and left carotid arteries. Their blood samples were also sent for adiponectin and leptin levels. The comparison was then made of Adiponectin-Leptin ratio as well carotid IMT between cases and controls. The results showed statistically high mean IMT of both right and left carotid arteries in atherosclerotic individuals as compared to normal ( $p=0.022$  and  $0.004$  respectively). Furthermore, Adiponectin-Leptin ratio was also found to be statistically higher in atherosclerotic individuals ( $p=0.001$ ).

In our study mean IMT of both right and left carotid arteries examined via carotid ultrasound were statistically high in atherosclerotic individuals as compared to healthy subjects {(Right carotid;  $0.83 \pm 0.23$  mm and  $0.63 \pm 0.16$  mm respectively;  $p=0.022$ ) (Left carotid;  $0.85 \pm 0.25$  mm and  $0.64 \pm 0.16$  mm respectively;  $p=0.004$ )}. In one large comparative study of 936 patients, ultrasound of carotid arteries was performed. These patients were classified into normal (controls) and atherosclerotic groups (cases) on the basis of IMT of their carotid arteries. There were statistically high mean IMT in atherosclerotic as compared to normal individuals. Even after adjusting the data for age, gender, and co-morbidities, statistically high differences were also observed between the cases and controls.<sup>22</sup> These results were nearly comparable to this study.

Although, the role of adipokines has been established as a cardiovascular risk factors, atherosclerosis and metabolic syndrome in literature<sup>7,8</sup>, the association of leptin to adiponectin ratio with atherosclerosis and healthy individuals is limitedly addressed. In this study, we also

encountered statistically higher adiponectin-leptin ratio in atherosclerotic patients in contrast to normal healthy individuals. In this study, we also encountered statistically higher adiponectin-leptin ratio in atherosclerotic patients in contrast to normal healthy individuals (43.82+72.73 and 0.05+0.04 respectively). The difference was statistically significant ( $p=0.001$ ).

None of the study in literature has evaluated the correlation of both right and left carotid artery IMT with adiponectin leptin ratio in atherosclerotic individuals. This study was unique in this regard as it evaluated this aspect. In this study, positive ( $r = 0.223$ ,  $p = 0.043$ ) correlation of left carotid IMT with adiponectin leptin ratio in atherosclerotic individuals were noted. However, weak positive correlation of right carotid IMT with adiponectin leptin ratio ( $r=0.099$ ,  $p = 0.380$ ) was encountered. Norata et al.<sup>23</sup> in their prospective study of 110 healthy individuals demonstrated positive correlation of IMT with adiponectin leptin ratio ( $r = 0.36$ ,  $p < 0.001$ ). The results are nearly comparable to this study.

## CONCLUSION

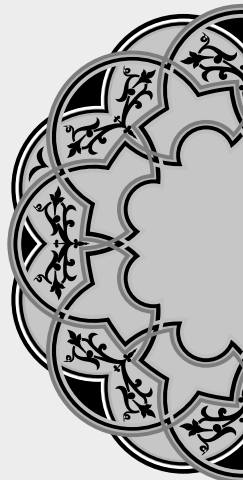
The plasma levels of Adiponectin and Leptin are significantly higher in atherosclerotic than normal individuals. The adiponectin/leptin ratio and Intima Media Thickness are substantially elevated in atherosclerotic as compared to normal individuals. In atherosclerotic individuals, positive association is noted between carotid IMT and adiponectin/leptin ratio.

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*“Even fools seem smart when they are quiet.”*

**Proverbs**

#### AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author-s Full Name	Contribution to the paper	Author=s Signature
1	Kausar Abbas Saldera	Conception and design, Critical revision of the article for important intellectual content	
2	Sanum Ali	Statistical expertise, Critical revision of the article for important intellectual content	
3	Ahsan Ashfaq	Drafting of the article	