The Association of *Chlamydia pneumonia* and *Helicobacter pylori* IgG Seropositivity With Omentin-1, Visfatin and Adiponectin Levels in Postmenopausal Women

Reza Ranjbar¹, Alireza Bolkheir², Katayoun Vahdat³, Majid Assadi³, Hossein Darabi³, and Iraj Nabipour⁴

¹Molecular Biology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
²Department of Infectious Diseases, The Persian Gulf Tropical Medicine Research Center, The Persian Gulf Biomedical Sciences Research Institute, Bushehr University of Medical Sciences, Bushehr, Iran
³The Persian Gulf Nuclear Medicine Research Center, The Persian Gulf Biomedical Sciences Research Institute, Bushehr University of Medical Sciences, Bushehr, Iran
⁴Department of Biochemistry, The Persian Gulf Marine Biotechnology Research Center, The Persian Gulf Biomedical Sciences Research Institute, Bushehr University of Medical Sciences, Bushehr, Iran

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Abstract- Some adipocytokines are cardioprotective or pro-inflammatory for the cardiovascular system. Chronic infection with *Chlamydia pneumonia* (*C. pneumoniae*) and *Helicobacter pylori* (*H. pylori*) has been also considered as novel risk factors for atherosclerosis. The main aim of the current population-based study is to investigate the potential link between circulating adipocytokines and *Chlamydia pneumonia* or *H. pylori* IgG seropositivity. A total of 250 healthy postmenopausal women who participated in a prospective cohort study were evaluated for IgG antibodies directed against *C. pneumoniae* and *H. pylori*. Omentin-1, visfatin, adiponectin, and high-sensitivity C-reactive protein were measured by highly specific enzyme-linked immunosorbent assay methods. The prevalence of IgG antibodies against *C. pneumoniae* and *H. pylori* among the studied population was 20.4% (51 women) and 57.2% (143 women), respectively. There were no significant differences in adipocytokine levels between *H. pylori* IgG seropositive and *H. pylori* seronegative subjects. Similar results for visfatin and omentin-1 were found when *C. pneumoniae* IgG seropositive were compared with *C. pneumoniae* IgG seronegative subjects. However, in general, linear model adjusted for age; body mass index and hs-CRP levels revealed asignificant difference between *C. pneumoniae* seropositive and *C. pneumoniae* seronegative subjects for circulating adiponectin. In conclusion, *C. pneumoniae* IgG seropositivity was associated with higher adiponectin levels in postmenopausal women. The elucidation of interaction mechanism of *C. pneumonia* and a cardioprotective adipocytokine (adiponectin) will be useful in future therapeutic strategies.

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Keywords: *Chlamydia pneumoniae; Helicobacter pylori; Adiponectin; Visfatin; Omentin-1*

Introduction

A growing body of clinical evidence has indicated that the increased size of adipocytes and chronic low-grade inflammation within the adipose tissue can alter the secretion and production of a variety of biologically active protein factors called adipocytokines (1).

Some adipocytokines have recently become the focus of research on the role of obesity in atherosclerosis because they have important complex interactions with endothelial cells, arterial smooth muscle cells, and macrophages in vessel walls (2,3).

Adiponectin is a good adipocytokine that has attracted great attention due to its anti-diabetic, anti-inflammatory, anti-thrombotic, and anti-atherogenic properties (4). It has been hypothesized that this adipocytokine may be a cardioprotective adipocytokine (5-7).
Omentin-1 is a novel 34 kDa adipocytokine, which not only regulates energy metabolism but also is implicated in the complex interactions between fat and heart (8). In fact, this adipocytokine is highly expressed and secreted by epicardial adipose tissue (9). The studies on the effect of recombinant omentin-1 on cardiomyocyte contractile function and insulin action, suggest that lower omentin-1 levels may be implicated in the pathogenesis of cardiac dysfunction in type 2 diabetes mellitus (10).

Visfatin, which is identical to the “pre-B-cell colony-enhancing 91 factor (PBEF1)” and “nicotinamide phosphoribosyltransferase (Nampt),” is secreted abundantly by the visceral fat of humans and mice and mimics the action of insulin (11). Visfatin was reported to be involved in cardiovascular diseases (12,3). However, whether this adipocytokine is a cardioprotecive or a risk factor for ischemic heart disease remains uncertain (14).

Chronic infection with C. pneumonia, H. pylori, and persistent viruses such as herpes simplex virus 1 (HSV-1) and cytomegalovirus (CMV) has been also considered as novel risk factors for atherosclerosis and the subsequent cardiovascular diseases (15-17). A growing body of evidence has demonstrated a contributory role for C. pneumonia, an obligate intracellular organism, in the pathogenesis of coronary artery disease, stroke, and peripheral atherosclerosis (18-20). Although the contributory role of $H.\text{ pylori}$ in the pathogenesis of atherosclerosis and ischemic heart disease remains enigmatic (21), this ubiquitous gram-negative bacterium has been shown to be associated with coronary artery disease and stroke (22,23). Chronic infection with $H.\text{ pylori}$ may be a risk factor for early coronary atherosclerosis regardless of traditional cardiovascular risk factors (24).

Despite the rapidly growing number of studies about the involvement of $C.\text{ pneumoniae}$ infection and $H.\text{ pylori}$ in cardiovascular diseases through the atherosclerotic changes in the vasculature that arise from the chronic inflammatory response (21,24), the pathogenic mechanisms are not fully understood.

Adipocytes like endothelial cells, monocytes, and hepatocytes produce inflammatory cytokines in response to infection with a range of different viral and bacterial organisms (25). The increased production of IL-6 and TNF-alpha by infected abdominal adipocytes and secondarily release of CRP by the liver may contribute to the initiation of atherosclerosis. In another aspect, the infection-induced inflammatory response may influence the adipocytes and alter the release of adipocytokines.

Theoretically, this alteration in the secretion of adipocytokines may lead to increase cardiovascular risk.

The main aim of the current population-based study is to investigate the potential link between circulating adipocytokines and $C.\text{ pneumonia}$ or $H.\text{ pylori}$ IgG seropositivity. To the best of our knowledge, the current study is the first to evaluate omentin-1 and visfatin in relation to chronic infection with $Chlamydia pneumoniae$ and $H.\text{ pylori}$.

Materials and Methods

Community sampling

In an extension arm of the Iranian Multicentral Osteoporosis Study, a community-based longitudinal study, an age-stratified random sample of postmenopausal women was selected from 13 clusters in Bushehr Port (the center of Bushehr province, which has the longest border with the Persian Gulf). All were community dwelling and ambulatory. The study design was described previously in detail (12). The participants in the current study were a subset of 250 postmenopausal women who participated in the extension arm of the Iranian Multicentral Osteoporosis Study. The study was approved by the medicaethical committee of Bushehr University of Medical Sciences, and written informed consent was obtained from all subjects.

Physical examinations

A stadiometer was used to measure the subjects’ height and weight. Heavy outer garments and shoes were removed before the participants’ height, and weight was measured. Their body mass index (BMI) was calculated. Waist circumference was defined at the midway level between the costal margins and the iliac crests. Hip circumference was measured at the level of the greater trochanters.

Laboratory measurements

A fasting blood sample was taken. All the samples were promptly centrifuged and separated, and analyses were carried out at the Persian Gulf Health Research Center.

CRP-reactive protein (CRP) was measured by using CRP HS enzyme-linked immunosorbent assay (ELISA) (DRG International), a highly sensitive (hs) CRP assay. The concentration of 0.1 mg/l for CRP was estimated to be the lowest detectable concentration in the CRP HS ELISA assay. According to the inter-assay coefficient of variation (CV)<20%, the functional sensitivity of CRP
measurement was determined to be 0.1 mg/l.

Serum omentin-1 concentrations were measured using manual omentin-1 (human) detection (ELISA kit [inteletin-1 (human) ELISA kit, Apotech Corporation, Switzerland]). The detection limit of the assay was 0.4 ng/mL (range 0.5 to 32 ng/mL). The mean intra-assay and inter-assay CVs of the omentin-1 assay were 4.51% to 7.4% and 4.19% to 9.27%, respectively. The antibodies used in this kit are specific to the measurement of natural and recombinant human omentin-1. To detect visfatin in the serum samples, commercially (Cat. No. V0523EK) available enzyme-linked immunosorbent assay kit (Adipo-Gen, Seoul, Korea) was used according to the manufacturer's instructions. The assay sensitivity for visfatin was 0.10 ng/mL; the intra- and inter-assay coefficients of variance were 3.8–5.5% and 6.4–9.5%, respectively.

To detect adiponectin in the serum samples, commercially (Cat. Q6No.AG-45A-0001EK-KI01) available enzyme-linked immunosorbent assay kits (AdipoGen, Incheon, Korea) were used according to the manufacturer's instructions. The limit of detection of the assay was 100 pg/mL; the intra- and inter-assay coefficients of variance were 2.9% to 3.8% and 2.8% to 5.5%, respectively.

IgG antibodies against C. pneumoniae were measured by a commercial test kit (DRG Instruments GmbH, Germany). The kit is based on an indirect solid-phase enzyme immunoassay with horseradish peroxidase as a marker enzyme; the positivity threshold was enzyme immuno-units>45. Sera were screened for IgG antibodies against H. pylori with an ELISA kit (RadimSpA, Italy), and the samples were considered positive when IgG values were higher than 30 RU/ml for H. pylori.

Statistical methods

Normal distribution of the data was controlled with the Kolmogorov-Smirnov test. The significance of the difference in the results of any two groups was determined by chi-square analysis using 2x2 contingency tables for categorical variables. A two-tailed t-test was used to compare the mean values across groups. We found that log transformation of adipocytokines and hs-CRP levels gave a better fit to a Gaussian distribution. The geometric mean for those biochemical variables was defined as the arithmetic mean of the log-transformed data±SD, raised to the power of 10.

The general linear model (GLM) univariate procedure was used for regression analysis and analysis of variance for one dependent variable (each adipocytokines levels) by IgG seropositivity for Chlamydia pneumonia or H. pylori (as the independent variable). The covariates were age, BMI and hs-CRP levels for GLM models of circulating adipocytokines.

Probability values<5% were considered statistically significant. All statistical analyses were performed using the PASW Statistics GradPack 18 (SPSS Inc., Chicago, IL).

Results

Table 1 shows the baseline characteristics of the studied postmenopausal women, stratified by IgG seropositivity against C. pneumoniae and H. pylori. The mean age (mean±SD) of the women was 58.87±8.02 years. The prevalence of IgG antibodies against C. pneumoniae and H. pylori among the studied population was 20.4% (51 women) and 57.2% (143 women), respectively.

There were no significant differences in age, anthropometric measures and adipocytokine levels between H. pylori IgG seropositive and H. pylori seronegative subjects (Table 1, P>0.05). Similar results were found when C. pneumoniae IgG seropositive were compared with C. pneumoniae IgG seronegative subjects, except for the significant difference in serum adiponectin levels.

Bivariate correlation analysis showed a correlation between circulating adiponectin and age (r=0.187, P=0.001), BMI (-0.160, P=0.004), and hsCRP levels (r=0.127, P=0.022). However, no significant correlations were found between omentin-1 and age, BMI, and hs-CRP levels (P>0.05). Visfatin levels were correlated with BMI (r=0.160, P=0.004) and hs-CRP levels (r=0.283, P<0.0001).

GLM analyses for omentin-1 and visfatin levels showed no significant differences between C. pneumoniae seropositive and C. pneumoniae seronegative subjects after adjustment for covariates (age, BMI, and hs-CRP levels). However, age-adjusted adiponectin levels were significantly higher in C. pneumoniae seropositive than C. pneumoniae seronegative subjects. This significant difference remained after further adjustment for BMI and hs-CRP levels (P=0.005, Table 2).

GLM analyses for omentin-1 and visfatin and adiponectin levels showed no significant differences between H. pylori seropositive and H. pylori seronegative women (P>0.05, Table 2).

Omentin-1, visfatin and adiponectin levels in postmenopausal women

Table 1. The baseline characteristics of the studied postmenopausal women, stratified by IgG seropositivity against *C. pneumoniae* and *H. pylori*

<table>
<thead>
<tr>
<th></th>
<th><em>C. pneumoniae</em> IgG</th>
<th><em>H. pylori</em> IgG</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Age, y</td>
<td>59.61±8.78</td>
<td>58.68±7.82</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>99.25±12.34</td>
<td>99.19±10.19</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>28.07±5.23</td>
<td>28.32±4.60</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.92±0.08</td>
<td>0.92±0.06</td>
</tr>
<tr>
<td>Omentin-1, ng/mL, *</td>
<td>10.78±2.55</td>
<td>11.29±2.19</td>
</tr>
<tr>
<td>Adiponectin, µg/mL,*</td>
<td>12.97±1.59</td>
<td>10.31±1.56</td>
</tr>
<tr>
<td>Visfatin, ng/mL,*</td>
<td>2.44±1.83</td>
<td>2.79±1.90</td>
</tr>
<tr>
<td>hs-CRP, mg/L,*</td>
<td>1.66±2.37</td>
<td>1.92±2.77</td>
</tr>
</tbody>
</table>

Data are given as means±SD
*Geometric mean±SD

Table 2. Age-, body mass index-, and hs-CRP adjusted adipocytokine levels by IgG seropositivity of *Chlamydia pneumoniae* and *H. pylori* in postmenopausal women in GLM analyses

<table>
<thead>
<tr>
<th></th>
<th><em>C. pneumoniae</em> IgG</th>
<th><em>H. pylori</em> IgG</th>
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<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Adiponectin, µg/mL</td>
<td>12.70±1.59</td>
<td>10.35±1.55</td>
</tr>
<tr>
<td>Omentin-1, ng/mL</td>
<td>10.78±2.55</td>
<td>11.22±2.18</td>
</tr>
<tr>
<td>Visfatin, ng/mL</td>
<td>2.41±1.77</td>
<td>2.77±1.90</td>
</tr>
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</table>

Data are geometric mean±standard deviation

Discussion

In the current study, we found that postmenopausal women with *C. pneumoniae* IgG positive antibodies had significantly higher levels of adiponectin compared with Chlamydia pneumonia IgG negative postmenopausal women. This association was independent of hsCRP and obesity measures.

*C. pneumoniae* has now been considered a novel risk factor for atherosclerosis since this organism was directly detected in atheroma (27,28). It has been shown that PPAR alpha and PPAR gamma pathways are involved in *C. pneumoniae*-induced macrophage-derived foam cell formation, a typical pathological feature of early atherosclerosis (29,30). Adiponectin can suppress lipid accumulation in macrophages and inhibit macrophage-derived foam cell formation (31). Therefore, the observed increased circulating levels of adiponectin in the current study may act as a counter-regulatory mechanism of attenuating detrimental effect of *C. pneumoniae* in atherosclerosis development via macrophage-derived foam cell formation.

Kim *et al.* investigated the change of PPAR gamma after the infection of the human coronary artery smooth muscle cells with *C. pneumoniae* (32). They found that *C. pneumoniae* could upregulate the expression of PPAR gamma mRNA and protein in vascular smooth muscle cells (32). As an important member of the nuclear receptor superfamily, PPAR gamma is a modulator of the inflammatory response in vessel walls (33,34).

PPAR gamma can induce the transcriptional activation of the adiponectin gene (35). It has been shown that PPAR-alpha activation not only reduced inflammation and the expression of macrophage-specific genes but also upregulated adiponectin receptors in white adipose tissue. Moreover, dual PPAR-alpha and PPAR-gamma activation increased adiponectin and its receptors (36). These experiments provide a plausible explanation for the observed significant association between *C. pneumoniae* IgG seropositivity and elevated adiponectin (as a good adipocytokine) levels. In fact, the observed higher circulating adiponectin in chronic
infection with C. pneumonia might be a physiological compensation and adaptation to protect vasculature from atherosclerosis development.

It has been reported that a range of viral and bacterial organisms are able to infect human adipocytes and induce inflammatory cytokines in vitro (25). Shi et al., demonstrated that C. pneumoniae could infect murine pre- and post-differentiated adipocytes and impair insulin signaling via an inflammatory pathway (37). However, none of the infective or inactivated microorganisms including C. pneumoniaeinduced significant changes in adiponectin production in adipocytes (25). Hence, positive association between C. pneumoniae and circulating adiponectin in our study might be beyond direct involvement of adipocytokines by this organism.

Recently, the effects of new C1q/TNF-related protein (cartonectin) with structural homologies to adiponectin on the adipokine secretion by adipocytes were studied (38,39). Further study is warranted to determine whether C. pneumoniae infection can contribute to cartonectin secretion because this novel adipokine is expressed in human adipocytes and regulated by metabolic and infection-related factors (39).

In addition, we did not find a similar association between C. pneumoniae IgG seropositivity and the other novel adipokines like omentin-1 and visfatin. Since there is no previously reported study in C. pneumoniae infection in relation to omentin-1 and visfatin in the medical literature, in agreement with the results of the current study, two independent researchers reported circulating adiponectin levels were not different between H. pylori positive and H. pylori negative patients (40,41). Similar levels of adiponectin in H. pylori IgG antibodies positive and negative in patients who underwent endoscopy for dyspepsia were reported by Ando et al., (40). However, eradication of H. pylori increased total and high molecular weight adiponectin levels in these patients (40). It has been hypothesized that it was modulation of gut microbiota by H. pylori eradication regimen responsible for the observed increased levels of adiponectin, in an H. pylori independent infectious process (42). We acknowledge several limitations in our study. We used IgG seropositivity against C. pneumoniae and H. pylori as a marker of prior infection. These serologic studies may not reflect persistent, chronic active or reinfection. Although the participants of the current study were healthy postmenopausal women who were randomly selected from a general population, the under or over estimating C. pneumoniae and H. pylori infections using serological tests was possible because of the difficulty in obtaining relevant clinical data in such setting. Currently, there is no valid marker to show the presence of chronic infection with C. pneumoniae seroepidemiological studies (43). However, the standardization and validation of nucleic acid amplification tests to show DNA evidence of infection may offer the potential for identifying currently infected patients in the future studies. Interestingly, it has been reported that there is a significant relationship between organism-specific DNA or antigens in coronary arteries obtained at autopsy and levels of pre-existing C. pneumoniae-specific IgG antibody titers (44). We had no longitudinal data to assess the longer-term association between C. pneumoniae seropositivity with adiponectin. Thus, the cross-sectional study design of our study did not allow us to examine the cumulative effects of adipokines and chronic C. pneumoniae over the course of the participants’ lives. Although this study is the first population-based study to investigate a link between C. pneumoniae or H. pylori seropositivity and adipokines, its findings should be confirmed in further human studies with larger samples.

In conclusion, C. pneumoniae IgG seropositivity was associated with higher adiponectin levels in postmenopausal women. This association should be validated in prospective studies. If confirmed, the knowledge of how the interaction between prior infections with pathogenic organisms involved in atherosclerosis with a cardioprotective adipokine (adiponectin) occurs will be useful in future therapeutic strategies. Undoubtedly, the elucidation of Chlamydia pneumoniae and adipokines interaction is a promising target for the treatment and prevention of cardiovascular diseases.

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References

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28. Kuo C, Campbell LA. Detection of Chlamydia


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