**Chlamydia pneumoniae** Infection in Diabetic Patients with Dyslipidemia

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*Chlamydia pneumoniae* is an obligate parasite capable of producing chronic infections. The severity of infection is closely related to hyperglycemia and changes in lipid profile. Since patients with Diabetes mellitus (DM) are at high risk of cardiovascular disease (CVD), the prevalence rate of specific *C. pneumonia* IgG / IgA antibodies, its association to the change in diabetic control HbA1c and lipid profile were estimated in 150 patients with DM and 50 healthy control subjects. *C. pneumoniae* (CPN) antibodies were estimated in 70% of DM patients using MIF and rDNA ELISA assays. 75 and 25% of patients were positive for CPN-IgG and IgA respectively using rDNA ELISA with prevalence rate 56.5 %, while 88 and 12% of patients were positive for CPN-IgG and IgA respectively using MIF assay which considered a gold standard method. There was significant (p=0.01) increase in the lipid profile except HDL-cholesterol (HDL-C) in diabetic patients with CPN-IgG antibodies compared to those having positive CPN-IgG antibodies. Significant positive correlations in BMI, Total cholesterol, triglyceride, LDL-C and negatively with HDL-C were reported in diabetic patients with varying *C. pneumoniae* antibodies titers. The data obtained suggested that, the change in lipid profile and HbA1c may play a pivotal role in the severity of *C. pneumoniae* infection. Consequently, the reduction of lipid profile may be a new therapeutic target against *C. pneumoniae* infection in diabetic patients.

**Key words:** rDNA LPS ELISA, lipid profile, Diabetes, HbA1c, *C. pneumoniae*.

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*Diabetes mellitus* is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both1. The increased morbidity and mortality of diabetic patients is mostly attributed to complications of the disease2. Chronic hyperglycemia leads to several events that promote structural changes in tissues and are associated with impaired wound healing3, higher susceptibility to infections4, and micro and macro vascular dysfunctions5.

Infection with *Chlamydia pneumoniae* (CPN) occurs worldwide, resulting in a 40-90% prevalence of serum antibody to the species in various populations6. Indirect evidence suggests that increased blood glucose due to DM may help sustain persistent *C. pneumoniae* infections to compensate for the extra energy load on infected cells. Chlamydial infections increase glucose consumption, lactate production, glutamate synthesis, glycogen accumulation, and an associated increase expression of the glucose transporter, GLUT-1, *in vitro*7. Conversely, diabetics are assumed to be very susceptible to infections with C. pneumonia. There may be an association between chronic *C. pneumoniae* infections and diabetic nephropathy based upon

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anti-*C. pneumoniae* serum IgG antibody titers in an ELISA. Some other studies have described associations between *Chlamydia pneumonia* seropositivity and the metabolic syndrome and dyslipidemia. Abnormal cholesterol and lipoproteins are major risk factors associated with atherosclerosis in diabetic and non diabetic patients. Atherosclerotic plaques contain foam cells and evidence of altered lipid composition. Animal models show that *C. pneumoniae* can induce atheromas and mimic inflammatory processes present in human atherosclerosis. Also, in previous cross-sectional study, a significant association between the presence of *C. pneumoniae*-specific IgG antibodies, elevated serum triglyceride, and lowered HDL cholesterol concentrations reported in a male population in Northern Finland.

Regarding the probable *C. pneumoniae* pathogenic severity in diabetic patients, the objective aim of this study is to investigate the possible role of serum lipids, BMI, HbA1c and their correlation to specific *C. pneumoniae* antibodies in diabetic patients.

**MATERIALS AND METHODS**

**Patients**

A total of 200 individuals recruited from the clinic of diabetic patients, Mansoura University Hospital, Mansoura, Egypt. Out of these 50 healthy individuals (25 men and 15 women, between 18 and 65 years old) with a mean age of 30±7.12 served as controls who attended for routine health check up at the hospital. None of the healthy control was taking any medicine or dietary supplement; they were selected after detailed physical examination and laboratory tests. A total of 150 of type 2 diabetes mellitus (DM2) (110 men and 40 women, aged from 20 to 64 years old) with a mean age of 45.9±4.6 were included in this study. The diagnosis of DM was based on the American Diabetes Association criteria for DM2 (fasting plasma glucose level higher than 126mg/dl and/or glucose level exceeding 200 mg/dl at 2 hours in the 75 g oral glucose tolerance test). Patients with type1 diabetes, smokers, anemia, overt complications of diabetes like nephropathy, neuropathy, retinopathy, obvious ischaemic heart disease (angina, myocardial infarction, and lead electrocardiogram abnormalities), HCV, HBV, chronic liver disease, hypothyroidism, overweight and obesity (BMI: ≥ 25 and ≥ 30 Kg/m2), and drugs (diuretics; oral contraceptives) were excluded from the study. All subjects completed a structured questionnaire with questions about demographic data, tobacco use and daily medication use. After 12 hrs fasting 5 ml of venous blood samples were collected in plain tubes, the samples were allowed to clot for half an hour following which a samples were centrifuged for 15 minutes at 2000 rpm. Samples were given a coded study identification number and were shipped frozen at -80°C until analysis. All serological and microbiological serum analysis was performed at clinical chemistry and microbiological laboratories, Mansoura University Hospital, Mansoura, Egypt. The demographics and baseline characteristics of patients and controls are presented in (Table 1). The study protocol was approved by ethical committee of Mansoura University Hospital, Mansoura, Egypt.

Serum samples were collected by standard procedures and were stored at 280°C prior to processing. From each patient with type 2 diabetes mellitus, nasopharyngeal specimens were collected with sterile cotton-tipped aluminum-shafted swabs and were suspended in 1.5 ml of Chlamydia transport medium (0.2 M sucrose phosphate [2SP]). Sputum samples were collected by standard procedures. **Laboratory assays for *C. pneumoniae* ELISA. Chlamydia-specific IgG and IgA antibodies**

ELISA. Chlamydia-specific IgG and IgA antibodies were detected by an rDNA LPS ELISA (Medac GmbH, Hamburg, Germany). The rDNA LPS ELISA may currently be the preferred tool for diagnosing acute respiratory Chlamydia infections in routine clinical practice. This ELISA includes a chemically pure structure of a recombinant LPS which contains a genusspecific epitope of the Chlamydia spp. pathogenic for humans 14-16. Initial serum dilutions for the detection of IgG, IgM, and IgA were 1:100, 1:50, and 1:50, respectively. Prior to the IgM determinations, IgG absorption was performed 17. Sera with optical density values exceeding 2.5 were retested with a 1:4 predilution. A twofold serially diluted standard serum sample
was used to calculate the log2 titer of the patients' samples. The IgG, IgM, and IgA cutoff values were calculated as prescribed by the manufacturer. The prevalence of Chlamydia IgG, IgM, and IgA antibodies was based on the following criteria: greater than or equal to the calculated cutoff 31.10, greater than or equal to the calculated cutoff 31.15, and greater than or equal to the calculated cutoff 31.10, respectively. Serological diagnosis of an acute Chlamydia infection was based on the ELISA results by using the following criteria: a threefold or greater increase in Chlamydia-specific IgG or IgA antibody titer, a twofold or greater change in the specific IgM titer, or a twofold increase in the specific IgG antibody titer in combination with a twofold increase in the specific IgA antibody titer.

**MIF assay**

The MIF assay described before, was used to measure *C. pneumoniae*-specific IgG, and IgA antibodies. Briefly, purified *C. pneumoniae* elementary antibodies (strain AR 39; Washington Research Foundation) were used to detect IgG, and IgA antibodies to *C. pneumoniae*. The prevalence of *C. pneumoniae*-specific IgG, and IgA antibodies was based on the presence of IgG at titers of ≥1:32, and IgA at titers of ≥1:32, respectively. Diagnosis of an acute *C. pneumoniae* infection was based on the following criteria: the presence of *C. pneumoniae*-specific IgM in either acute- or convalescent-phase serum or a fourfold or greater increase in the *C. pneumoniae*-specific IgG and/or IgA antibody titer between the acute- and convalescent phase serum samples.

**Analysis of Blood sugar and Glycated Hemoglobin (HbA1c):**

Blood glucose was measured by Glucose Oxidase and Peroxidase (GOD-POD) colorimetric method (Quanti Chrom. TM Glucose Assay Kit, D1G1-100, BioAssay Systems, Hayward, CA 94545, USA). HbA1c was measured with the quantitative sandwich enzyme immunoassay technique (Human HbA1c ELISA kit, Cat. No. E0897h, eiaab.co).

**Analysis of serum lipid profile**

Serum total cholesterol levels was determined by enzymatic (CHOD-PAP) colorimetric method and triglycerides by enzymatic (GPO-PAP) method as previously described. HDL-cholesterol was estimated using precipitant method, and LDL cholesterol by Friedewald formula.

**Statistical analysis**

Variables were summarized by standard descriptive statistics and expressed as mean ±SD. After entering the data to a masked database the analysis was performed by an unaware statistician, using the Statistical Package of Social Sciences (SPSS) version 11.5.0 (SPSS, Inc. Chicago, Illinois). The Student-test, chi-square, and ANOVA were used for analysis of quantitative and qualitative data, respectively. The significance level was set at 0.05.

**RESULTS**

One hundred and fifty patients with type 2 diabetes mellitus (110 men and 40 women), with a mean age of 45.9±4.6 and fifty healthy controls (25

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**Table 1. Clinical and demographic characteristics of the study population**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Controls</th>
<th>DM2 Patients</th>
<th>*P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>50</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td>Age ( Year) (mean ± SD)</td>
<td>30 ±7.12</td>
<td>45.9±4.6</td>
<td>**0.001</td>
</tr>
<tr>
<td>Gender (Male/Female)</td>
<td>25/15</td>
<td>110/40</td>
<td></td>
</tr>
<tr>
<td>BMI (mean ± SD)</td>
<td>17.6±3.7</td>
<td>21.8±4.1</td>
<td>** 0.001</td>
</tr>
<tr>
<td>Time since the DM onset (n, %, mean ± SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 10 years</td>
<td>45 (30.0), 8.13±4.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥10 years</td>
<td>105 (70.0), 13.13±5.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medication for control of DM ( n, %)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoglycemic</td>
<td>97 (64.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>35 (23.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoglycemic and Insulin</td>
<td>18 (12.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P for controls vs Diabetic patients; (Student- t test), * P<0.05; **P<0.01; SD: Standard deviation; DM2: type 2 diabetes mellitus; BMI: Body mass index
Table 3. Correlation between Chlamydia-specific antibodies determined by rDNA LPS ELISA and MIF assays of 150 patients with type 2 diabetes.

<table>
<thead>
<tr>
<th>Study population</th>
<th>No. of subjects</th>
<th>Assay</th>
<th>Prevalence (% of patients seropositive CPN antibodies)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM2</td>
<td>150</td>
<td>rDNA LPS ELISA</td>
<td>57.5 (%) IgG, 25% IgA</td>
</tr>
<tr>
<td>Control</td>
<td>50</td>
<td>MIF</td>
<td>65% (%)</td>
</tr>
</tbody>
</table>

*Correlation coefficient, 0.902; P = 0.005 (by chi-square test).
Table 5. Correlation coefficients of HbA1c and sugar compared to other variables in diabetic patients with or without Chlamydia pneumoniae IgG or IgA Antibodies.

<table>
<thead>
<tr>
<th>Variables</th>
<th>DM2 patients with CPN Negative antibodies</th>
<th>DM2 Patients with CPN Positive antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HbA1c</td>
<td>Sugar</td>
</tr>
<tr>
<td>No.</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>0.19*</td>
<td>0.795*</td>
</tr>
<tr>
<td>T.Chol., mmol/L</td>
<td>0.18*</td>
<td>0.956*</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>0.258*</td>
<td>0.382*</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>-0.26*</td>
<td>-0.46**</td>
</tr>
<tr>
<td>LDL- C, mmol/L</td>
<td>0.203*</td>
<td>0.672*</td>
</tr>
</tbody>
</table>

*p HbA1c or sugar vs other variables in Diabetic patients with positive or negative CPN antibodies; * p<0.05; **p<0.01; SD: Standard deviation; DM2: type 2 diabetes mellitus; CPN: Chlamydia. Pneumoniae; HbA1c: Glycated Hemoglobin Ac1; T- chol.: total cholesterol; TG: triglycerides; HDL: high density lipoprotein; LDL: Low Density Lipoprotein.

Diabetes mellitus (DM) affects more than 100 million people worldwide and its incidence and prevalence are still rising with higher risk of cardiovascular disease. Cross-sectional and longitudinal studies demonstrate significant positive correlations between measures of plasma glucose and corresponding plasma lipids.

Diabetic patients with accompanied dyslipidemia are soft targets of cardiovascular deaths. An early intervention to normalize circulating lipids has been shown to reduce cardiovascular complications and mortality.

The purpose of this study was to determine the correlation between Chlamydia pneumoniae (CPN) infection and serum lipids in diabetic patients. The most important predictor of change in plasma lipids was the change in HbA1c and glucose levels.

In our study, significant higher levels of total cholesterol, TG, LDL-C levels and decrease in HDL-C were reported in diabetic patients compared to healthy volunteers. The data obtained matched with other studies which showed that elevated concentration of LDL-C in the blood are powerful risk factors for coronary heart disease in patients with diabetes.

In our study, BMI, T-Cholesterol, triglycerides and LDL correlated positively and HDL-C negatively with HbA1c and glucose concentration in diabetic patients with or without anti- Chlamydia pneumoniae antibodies (Table 5).

DISCUSSION

Diabetes mellitus (DM) affects more than 100 million people worldwide and its incidence and prevalence are still rising with higher risk of cardiovascular disease. Cross-sectional and longitudinal studies demonstrate significant positive correlations between measures of plasma glucose and corresponding plasma lipids.

Diabetic patients with accompanied dyslipidemia are soft targets of cardiovascular deaths. An early intervention to normalize circulating lipids has been shown to reduce cardiovascular complications and mortality.

The purpose of this study was to determine the correlation between Chlamydia pneumoniae (CPN) infection and serum lipids in diabetic patients. The most important predictor of change in plasma lipids was the change in HbA1c and glucose levels.
proinflammatory cytokines. This condition with impaired immune-reactivity makes DM patients prone to microbial infections. The development of atherosclerotic plaques in diabetic patients may be related to the presence of C. pneumoniae in blood.

In our study, C. pneumoniae antibodies were estimated in 70% of diabetic patients. Out of them, 40% of patients were positive for IgG and 30% with IgA antibodies. C. pneumoniae antibodies were significantly (P < 0.001) correlated with both serum glucose and HbA1C. It was reported that C. pneumonia infection is ubiquitous, with an antibody prevalence of 50% by age 20 and 70% to 80% at age 60 to 70. Recently, it was reported that C. pneumoniae antibody positivity was significantly more common in patients of poor metabolic control (HbA1c>9%) versus patients in good metabolic control (HbA1c<7%). These suggest an increased risk of C. pneumoniae infection in diabetic patients. The development of diabetes might be a possible factor for promoting C. pneumoniae dissemination.

This study indicates that the severity of C. pneumoniae infection is associated with the change in lipid profile. In diabetic patients whereas, the atherogenic effects of Chlamydia are claimed to be dependent on serum cholesterol and change in lipid metabolism.

CONCLUSION

The change in lipid profile and HbA1c play a pivotal role in the severity of C. pneumoniae infection in diabetic patients. So, the reduction of plasma lipid profile may be a new therapeutic target against C. pneumoniae infection.

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