Placental infection with *Chlamydia pneumoniae* and intrauterine growth restriction

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Abstract

**Background:** The concept that low birth weight infants are more predisposed to coronary artery disease (CAD) in adulthood has been studied extensively. Although many infectious agents have been associated with intrauterine growth restriction (IUGR), *Chlamydia pneumoniae* an organism implicated in CAD has not been investigated. It was our aim to assess whether *C*. *pneumoniae* DNA is present in placental tissue and whether its detection is associated with IUGR. **Methods:** Fifty-nine pregnant women were studied: 32 women had an uncomplicated pregnancy with no antenatal or post-natal evidence of IUGR. Twenty-seven women had pregnancies with ultrasonographically demonstrated IUGR, defined as foetal abdominal circumference measuring less than 2 S.D.s from the mean for gestational age. At the time of delivery, maternal blood and placental tissue samples were obtained. Placental samples were taken from four sites centrally and peripherally on the maternal and foetal side of the placentas and tested by nested polymerase chain reaction for *C*. *pneumoniae* DNA. IgG antibodies to *C*. *pneumoniae* were measured using microimmunofluorescence. **Results:** *C*. *pneumoniae* DNA was detected in 44% of the placental tissue but there was no difference in the prevalence of bacterial DNA between the control and the low birth weight group (\(P=0.58\)). Additionally *C*. *pneumoniae* seropositivity did not differ between the index and control groups (78 vs. 70%, \(P=0.44\)). **Conclusions:** *C*. *pneumoniae* is present in placental tissue. Its presence however does not correlate with IUGR. Similarly, maternal *C*. *pneumoniae* seropositivity is not related to low birth weight. Thus *C*. *pneumoniae* infection is unlikely to play a role in the pathogenesis of IUGR.

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**Keywords:** Atherosclerosis; Infection/inflammation; Congenital defects; Coronary circulation; Coronary disease

1. Introduction

Epidemiological and experimental studies have shown that retarded foetal growth can lead to serious perinatal complications such as stillbirth, increased morbidity and disorders extending well beyond childhood, including coronary artery disease, hypertension and diabetes mellitus [1,2]. Foetal growth is subject to genetic variation but the intrauterine environment also exerts a dominant influence. Although restricted growth has been linked to a large number of causes, including foetal and placental abnormalities [3–5], limited supply of nutrients [6] and infectious diseases [7–9], in most cases the aetiology remains unknown.

Over the past decade there has been a resurgence in the hypothesis that infection could play a pathogenic role in vascular disease. Experimental and clinical studies suggest that *Chlamydia pneumoniae* is one of the most plausible candidates for a role in atherogenesis [10]. *C*. *pneumoniae* is found in diseased vascular tissue and immunohistochemical as well as molecular studies have shown that the organism is associated with the extent and severity of
cardiovascular disease [11–13]. Infection with *C. pneumoniae* is widespread with 70% of adults found to be seropositive [14,15]. In addition recent information suggests that circulating leukocytes may act as reservoirs of bacteria in healthy individuals [16,17].

The tropism of *C. pneumoniae* and its prevalence in the general population led us to consider the placenta as a possible site of infection during pregnancy. Low birth weight may be a consequence of such an infection. A member of the same genus, *C. trachomatis*, is the most prevalent agent associated with sexually transmitted infections and linked to an increased incidence of stillbirth, prematurity and low birth weight [18,19]. The aim of this study was therefore to assess the relationship between seropositivity to *C. pneumoniae* and placental detection of the organism and intrauterine growth restriction (IUGR). Such an infection may explain the predisposition of low birth weight infants to coronary artery disease (CAD) in later life. To our knowledge there have been no reported studies looking at such an association.

2. Methods

This investigation conforms with the principles outlined in the Declaration of Helsinki (Cardiovascular Research 1997;35:2–3).

2.1. Patients studied

Fifty-nine patients from a district general hospital obstetric department in the North West of England were studied. Patients were allocated into index or control cases. Thirty-two control cases were identified on the basis of having an uncomplicated pregnancy with no antenatal or postnatal evidence of IUGR and delivery of a healthy, appropriate for gestational age-sized foetus, at or near term. The 27 index cases consisted of patients with ultrasonographically demonstrated IUGR, defined as foetal abdominal circumference measuring less than 2 standard deviations (S.D.s) from the mean for gestational age. Demographic and clinical data were recorded from all patients, including previous obstetric history and history of smoking.

At the time of delivery, maternal blood and placental tissue samples were obtained and the birth weight of the baby was recorded. No gross lesions were observed on the placentas and macroscopic examination revealed no evidence of infarcts. Placental samples were taken from four sites centrally and peripherally on the maternal and foetal side of the organs. The placental tissue was then immediately snap frozen and stored in liquid nitrogen until transfer to St. George’s Hospital Medical School for analysis. The study was approved by the local ethics committee and all patients gave written informed consent before study entry.

2.2. Serological assays

The *Chlamydia* micro-immunofluorescence assay (MIF) was used for assessment of antibody levels to *C. pneumoniae* according to the manufacturer’s instructions (MRL Diagnostics). Antigen extracts from *C. trachomatis* and yolk sac were used as controls. Sera were initially screened at a dilution of 1/16 and samples that reacted positively with *C. pneumoniae* were re-tested using serial twofold dilutions. The final dilution of antibody to show reactivity with *C. pneumoniae* in the absence of reaction with the control antigens was recorded as the antibody titre with anti-*C. pneumoniae* antibody titres ≥1/16 considered to be seropositive.

2.3. Polymerase chain reaction (PCR)

DNA was extracted from placental tissue using Qiagen DNA mini kit (Qiagen, UK), according to manufacturer’s instructions and subjected to nested PCR for amplification of the *OmpA* gene of *C. pneumoniae* [20]. DNA extracted from Hep2 cells infected with the TW183 strain of *C. pneumoniae* was used as positive control. The sequence of primers used for amplification, the size of the amplimers and the reaction conditions are shown in Table 1. All first round amplification reactions were performed using 1 μg

<table>
<thead>
<tr>
<th>Target</th>
<th>Primers</th>
<th>Amplicon size (bp)</th>
<th>No. of cycles</th>
<th>MgCl₂ (mM)</th>
<th>Annealing temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OmpA</td>
<td>First round primers</td>
<td>333</td>
<td>40</td>
<td>1.5</td>
<td>Step-down 65–55</td>
</tr>
<tr>
<td></td>
<td>CP1 5’-TTACAAGCCTGCTGTAGG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CP2 5’-GCATCCCAATGTTGACGC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nested primers</td>
<td>207</td>
<td>30</td>
<td>1.5</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>CPC 5’-TTATTAATTTGATGGTACAAATA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CPD 5’-ATCTACGGCAGTAGTATGTT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myoglobin</td>
<td>5’-AACATGACAGGTCCCTTTGG</td>
<td>439</td>
<td>35</td>
<td>1.5</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>5’-CATGGCCAGTCTGAA</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
of DNA in 50 μl reaction volumes. A 1-μl volume of the product was subsequently used in nested PCRs.

2.4. Myoglobin PCR

Amplification of the myoglobin gene was used as housekeeping control [21]. Reactions were performed in 50-μl volumes using 100 ng of placental DNA. The sequence of primers used for amplification and the reaction conditions are shown in Table 1. The presence of the 439 base pair (bp) product confirmed integrity of the DNA extracted from the placenta.

2.5. Statistical analysis

Results for normally distributed variables are expressed as mean (S.D.), continuous variables with non-normal distribution are presented as mean (interquartile range) and categorical data are expressed as percentages. Continuous variables were analysed using unpaired t-tests and Mann–Whitney U-tests where appropriate. Categorical data and proportions were analysed using chi-square or Fisher’s exact test when required. A P value <0.05 was considered statistically significant. The SPSS 7.5 statistical software package was used for all statistical calculations.

3. Results

3.1. Clinical and demographic characteristics

Clinical and demographic characteristics of index and control cases are presented in Table 2. As per study design, index cases had significantly lower birth weights and shorter gestational ages than controls. There were no significant differences in maternal age and smoking habits between the two groups. A higher proportion of the index cases were multigravida, although this was not statistically significant.

Table 2
Clinical and demographic data of control and index case groups

<table>
<thead>
<tr>
<th></th>
<th>Control cases (n=32)</th>
<th>Index cases (n=27)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age, years</td>
<td>24±5</td>
<td>25±5</td>
<td>0.30</td>
</tr>
<tr>
<td>Parity</td>
<td>0 (0–4)</td>
<td>1 (0–4)</td>
<td>0.154</td>
</tr>
<tr>
<td>Gestational age, weeks</td>
<td>39±1</td>
<td>38±2</td>
<td>0.001</td>
</tr>
<tr>
<td>Birth weight, kg</td>
<td>3.4±0.4</td>
<td>2.3±0.4</td>
<td>0.000</td>
</tr>
<tr>
<td>C. pneumoniae seropositive, n (%)</td>
<td>25 (78)</td>
<td>19 (70)</td>
<td>0.44</td>
</tr>
<tr>
<td>Placenta C. pneumoniae DNA positive, n (%)</td>
<td>14 (44)</td>
<td>12 (44)</td>
<td>0.58</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoking</td>
<td>15 (47)</td>
<td>14 (52)</td>
<td>0.77</td>
</tr>
<tr>
<td>Non smoking</td>
<td>16 (50)</td>
<td>13 (48)</td>
<td></td>
</tr>
</tbody>
</table>

3.2. C. pneumoniae and low birth weight

There were no significant differences in the prevalence of positive C. pneumoniae serology or the titres of antibody between the control group and low birth weight group. 78% of the control population had antibodies to C. pneumoniae with a median titre of 1 in 128 whilst 70% of the index group were seropositive and had a median antibody titre of 1 in 64 (P=0.44). Nested PCR was performed on 236 separate placental tissue samples, as each patient had four placental samples taken centrally and peripherally from the maternal and foetal sides of the placenta. In all 236 samples the presence of intact DNA was confirmed by successful amplification of the myoglobin gene (data not shown). C. pneumoniae DNA was detected in 26 placental tissue samples 12 of which were in the index cases (44%) and 14 (44%) in the control group. Results for normally distributed variables are expressed as mean (S.D.), continuous variables with non-normal distribution are presented as mean (interquartile range) and categorical data are expressed as percentages. Continuous variables were analysed using unpaired t-tests and Mann–Whitney U-tests where appropriate. Categorical data and proportions were analysed using chi-square or Fisher’s exact test when required. A P value <0.05 was considered statistically significant. The SPSS 7.5 statistical software package was used for all statistical calculations.

4. Discussion

We have shown for the first time that placental tissue may harbour C. pneumoniae DNA. Encounter of this organism, implicated in coronary artery disease, may therefore occur during gestation. Previous studies using immunohistochemical examination methods on formalin fixed tissue have failed to identify C. pneumoniae proteins in human placenta [22]. The use of nested PCR provided the advantage of sensitivity and confirmed our hypothesis that circulating organisms may be deposited in this tissue.

No significant differences in the prevalence of infection were observed between pregnancies resulting in the delivery of growth restricted or normal for gestational age
Table 3
Clinical data of placental \textit{C. pneumoniae} positive and negative patients

<table>
<thead>
<tr>
<th></th>
<th>\textit{C. pneumoniae} DNA positive (n=27)</th>
<th>\textit{C. pneumoniae} DNA negative (n=32)</th>
<th>\textit{P} value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age, years (mean±S.D.)</td>
<td>24±5</td>
<td>25±5</td>
<td>0.81</td>
</tr>
<tr>
<td>Parity</td>
<td>1 (0–4)</td>
<td>1 (0–4)</td>
<td>0.80</td>
</tr>
<tr>
<td>Gestational age, weeks (mean±S.D.)</td>
<td>39±2</td>
<td>38±2</td>
<td>0.45</td>
</tr>
<tr>
<td>Birth weight, kg (mean±S.D.)</td>
<td>3.0±0.5</td>
<td>2.8±0.8</td>
<td>0.56</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>17 (63)</td>
<td>13 (41)</td>
<td>0.09</td>
</tr>
<tr>
<td>\textit{C. pneumoniae} seropositive, n (%)</td>
<td>21 (78)</td>
<td>23 (72)</td>
<td>0.50</td>
</tr>
</tbody>
</table>

infants. Using microimmunofluorescence methods to measure \textit{C. pneumoniae} IgG levels, no differences were found in the titres of antibody in the maternal serum obtained from the two study groups. PCR amplification methods found 44% of IUGR and 44% of control placentas to be positive for chlamydial DNA. This proportion agrees closely with reports that 40% of healthy blood donors may have circulating \textit{C. pneumoniae} DNA [17]. It is likely that maternal leukocytes infiltrating the placenta may be responsible for carrying the organism to this tissue. Whether the bacterial DNA found in placenta is always associated with maternal leukocytes or on occasion reflects infection of the placental cells is at present uncertain.

The high reported prevalence of \textit{C. pneumoniae} infection in the population is of consequence. The present study indicates that placental colonisation occurs during the gestational period. Whether the DNA found in this tissue represents organism capable of replicating is uncertain and in what proportion of cases if any the organisms cross the placenta to enter the foetal circulation is also unknown. Although we have shown no relationship between IUGR and placental infection, infection of the foetus may be a more relevant parameter to assess. Detection of \textit{C. pneumoniae} specific IgM class antibodies in the cord blood or the presence of bacterial DNA in leukocytes of foetal origin will identify the infected cases. Most primary bacterial and viral infections are detrimental to the foetal health and can result in severe sequela. Primary infections however, occur in a small proportion of pregnancies. Infection in the presence of protective maternal IgG may be a frequent event.

Placental infection with \textit{C. pneumoniae}, as shown in the present study, may have clinical implications. Foetal infection may occur more frequently than previously considered. Agents capable of residing in vascular tissue such as \textit{C. pneumoniae}, should be considered in the pathogenesis of cardiovascular disease, predisposition to which may start before birth.

Acknowledgements

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References


