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CHLAMYDOPHILA PSITTACI SEROPOSITIVITY AND SERUM LEVELS OF SOLUBLE INTERCELLULAR ADHESION MOLECULE-1 IN FARMERS

Concettina Fenga[1], Anna Cacciola[1], Venerando Rapisarda[1], Giovanna Spatari[1], Sebastiano Calimeri[2], Paola Lucia Minciullo[3], Giuseppe Di Pasquale[4], Sebastiano Gangemi[3]

[1] Section of Occupational Health, Department of Social and Environmental Medicine, University of Messina, Italy
[2] Department of Medical Veterinary Sciences, University of Messina, Italy
[3] School and Division of Allergy and Clinical Immunology, Department of Human Pathology, University of Messina, Italy
[4] Department of Pediatrics Science and Medical Surgery, University of Messina, Italy

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Abstract: *Chlamydophila* infection is known as an occupational hazard to veterinarians, farmers, poultry workers. Serum levels of the soluble Intercellular Adhesion Molecule-1 (sICAM-1), is associated with *C.* seropositivity. Since no data about ICAM-1 levels and *C. psittaci* infection are known, the aim of this work was to assess if chronic persistent *C. psittaci* infection constantly stimulates the expression of sICAM-1, independent of the characteristic symptoms of ornithosis. *C. psittaci* seropositivity and serum concentrations of sICAM-1 were investigated in 30 farmers and 20 age-matched healthy public employees as controls. Increased serum sICAM-1 levels were found in the group of farmers exposed to infectious risk compared to controls, and the serum concentrations of sICAM-1 was significantly correlated with a high IgG titre against *C. psittaci*. It is therefore possible to suggest a sICAM-1 measurement for use as a tool to verify the development of *C. psittaci* chronic infection in an occupational setting.

Address for correspondence: Concettina Fenga, Dipartimento di Medicina Sociale del Territorio, Sezione di Medicina del Lavoro, Policlinico Universitario – Pad. H, Via Consolare Valeria – Gazzi, 95024 Messina (Italy). E-mail: cfenga@unime.it

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INTRODUCTION

The characteristic feature of *Chlamydophila* is its tendency to cause chronic infections that are associated with a high plasmatic antibody titer. This condition has been shown in veterinarians, farmers, and poultry workers who are occupationally exposed during the management of animals [4, 8, 10, 12, 14, 16].

Several reseachers performed studies in order to identify plasmatic substances that could represent markers of persistent inflammation reaction, that often is associated with the development of a chronic *Chlamydophila* infection [5, 9, 13].

It is known that serum levels of the soluble Intercellular Adhesion Molecule-1 (sICAM-1) are associated with *Chlamydophila* seropositivity; this association is more evident in subjects with a high titer of *Chlamydophila* antibodies [7]. Enhanced expression of sICAM-1 in *Chlamydophila* infection is associated with increased rolling, adhesion, and transmigration of leukocytes and monocytes, which could sustain chronic inflammation independent of the characteristic symptoms of ornithosis, such as fever, chills, headache, myalgia [1, 6, 11, 15].

No data about ICAM-1 levels and *C. psittaci* infection are known. Therefore, the aim of this work was to assess if chronic persistent *C. psittaci* infection constantly stimulates the expression of sICAM-1.

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MATERIAL AND METHODS

The association between *C. psittaci* seropositivity and serum concentrations of sICAM-1 was investigated in a group of 30 caucasian male farmers aged 32 ± 7.78 years, with a length of employment of 8.27 ± 2.14 years, who worked in 3 cattle and pig farms located in eastern Sicily. None of them used any respiratory protection devices at work.

The control group consisted of 20 age-matched healthy public employees.

All subjects gave their written informed consent to participate in the study. They were interviewed by an occupational physician, who collected information about socio-demographic characteristics, occupational and clinical history, with particular regard to flu-like symptoms in the previous 6 months.

Venous blood (10 ml) was collected and allowed to clot at room temperature for 2 h; serum was separated by centrifugation at 200 xg for 15 min in a 4235 A centrifuge (ALC Int. S.r.l., Milan, Italy), and stored at –80°C until use.

Sera from the 30 farmers and 20 controls were tested for IgA, IgG and IgM directed against *C. psittaci* using a microimmunofluorescent (MIF) test kit (Servibio – France).

Serum specimens were screened at 1:16 dilution (IgA, IgG and IgM) and titrated to end point; an IgG titre of ≥ 1:512 or IgA and IgM antibody titers ≥ 1:16 were considered indicative of possible acute *C. psittaci* infection. Subjects with IgG titers of > 1:16 or < 1:512 were defined as seropositive and those with IgG titers < 1:16 as seronegative [17].

To rule out the presence of antibody cross-reactivity, testing was also performed for *C. trachomatis* and *C. pneumonie*. Serum samples were considered positive if they reacted only with *C. psittaci* antigen or with the other *Chlamydophila* types, but showed the highest grade of reaction with *C. psittaci* antigen.

sICAM-1 measurement. Serum sICAM-1 levels were assayed by an immunoenzymatic method (Human sICAM-1, ELISA-kits, R&D System, SPACE Import-Export, Milan, Italy), whose detection threshold was <0.35 ng/ml.

Statistical analysis. Data analysis was performed using the SPSS-PC software (SPSS, Italy). The data were summarized with the mean as a measure of central tendency and standard deviation as a measure of dispersion. The Student-Newman-Keults test was used to compare 2 means and the chi-square test (χ^2) for frequency analysis. The correlation among study parameters was analyzed using Spearman's rank coefficient. Significance was established at $p \leq 0.05$.

RESULTS

Socio-demographic, clinical characteristics and seroprevalence for *C. psittaci* are summarized in Table 1. There were no significant differences between the group under study and the control group in age, gender, smoking status, alcohol consumption and clinical characteristics. Length of employment was significantly different between the two groups (study group *vs.* control group: 2.97 ± 1.67 *vs.* 6.15 ± 2.16 $p < 0.05$).

Of the 30 farmers, 14 (46.67%) were seronegative (IgG ≤ 1:16) and 16 (53.33%) were seropositive (IgG > 1:16). All control subjects were seronegative (IgG ≤ 1:16).

On the basis of antibody titre, the 16 seropositive subjects were divided in: 1 (6.25%) subject with antibody titre

Table 1. Socio-demographic, clinical characteristics and seropositivity for *C. psittaci* and plasmatic level of sICAM1 of the study group (n = 30) and control group (n = 20).

		Study group	Control group	p<
Age	M ± SD	36.52 ± 8.16	38.45 ± 7.12	NS
Gender – males	%	100	100	
Smoking status (10 sig/day)	n (%)	12 (40)	15 (75)	NS
Alcohol consumption (1–2 glasses of wine a day)	%	100	100	
Systemic diseases		none	none	
Flu-like symptoms in last 6 month (fever, chills, headache, myalgia)		none	none	NS
Lengh of employment	M ± SD	2.97 ± 1.67	6.15 ± 2.16	0.05
Residence				
Rural areas (Messina)	n (%)	30 (100)		
Cities (Messina)	n (%)		20 (100)	
Hobbies in contact with animals		none	none	
Lenght of employement				
Rearing	n (%)	30 (100)		
Agriculture	n (%)	none	5 (25)	
Craftsmanship	n (%)		5 (25)	
Other	n (%)		10 (50)	
Seroprevalence for *C. pisittaci*				
Seronegative (IgG < 1:16)	n (%)	14 (46.67)	20 (100)	
Sieropositive (IgG > 1:16)	n (%)	16 (53.33)		
1:16 < IgG < 1:32		4		
1:32 < IgG < 1:64		5		
1:64 < IgG < 1:128		3		
1:128 < IgG < 1:256		4		
IgA and/or IgM		absent	absent	
Plasmatic level of sICAM-1 ng/ml	M ± SD	285.589 ± 72.194	222.366 ± 55.014	0.05

M – mean, SD – standard deviation, NS – not significant

Table 2. Plasmatic level of sICAM-1 of the group-1 (subjects with high antibody titer 1:64 < IgG < 1:256) and group-2 (subjects with low antibody titer 1:16 < IgG < 1:64).

	Group-1 (1:64 < IgG < 256) (n = 16)	Group-2 (1:16 < IgG < 1:64) (n = 14)	p<
Plasmatic level of sICAM-1 ng/ml (M ± SD)	323.934 ± 50.842	220.221 ± 52.169	0.01

M – mean, SD – standard deviation

Table 3. Correlation between antibody titres and plasma concentration of sICAM-1 in farmers and control group.

	Correlation (r)	Significance (p)
Farmers	0.64	0.05
Control group	0.25	not significant.

of 1:16 < IgG ≤ 1:32; 8 (50%) with antibody titer of 1:32 < IgG ≤ 1:64; 3 (18.75%) with antibody titre of 1:64 < IgG ≤ 1:128, and 4 (25%) with antibody titre of 1:128 < IgG ≤ 1:256. IgA and/or IgM antibodies were absent in the 2 groups (Tab. 1).

Serum levels of sICAM-1 were significantly higher in farmers than in control subjects (285.589 ± 72.194 ng/ml *vs.* 222.366 ± 55.014 ng/ml, $p < 0.05$).

Table 2 shows plasma concentrations of sICAM-1 in 2 groups categorized by high seropositivity (group 1 n = 16 1:64 < IgG < 256) and low seropositivity (group 2 n = 14 1:16 < IgG < 1:64) for *C. psittaci*. Plasma concentration of sICAM-1 was significantly higher in group 1 than in group 2. The relationship between plasma concentration of sICAM-1 and seropositivity for IgG was analysed. IgG index, as a continuous variable, showed a significant positive association with plasma sICAM1 ($r = 0.64$, $p < 0.05$) (Tab. 3).

DISCUSSION

The present study shows significantly higher sICAM-1 serum levels in subjects with seropositivity for *C. psittaci* compared to those who were seronegative. Moreover, in order to better define the correlation between antibody titer and serum concentration of sICAM-1, serum level of sICAM-1 was analyzed in the group with a higher indices of antibody for *Chlamydophila* infection, compared to serum level of sICAM in those subjects with a lower antibody titer, showing a significantly higher serum level of sICAM-1 in the former group. This finding, together with the result of a linear association between antibody indices and plasma concentrations of sICAM-1, may indicate that persistent *C. psittaci* infection is associated with an increase in serum levels of sICAM. It has been suggested that a higher titer of *Chlamydophila* antibody reflects chronic and persistent *Chlamydophila* infection independent of the characteristic symptoms of ornithosis, such as fever, chills, headache, myalgia [7].

ICAM-1 induces a persistent phlogistic reaction characterized by a continuos recruitment of leucocytes, monocytes and macrophages to the microvascular endothelium; it also increases cell interactions, such as limphocyte-epithelial cell contact and cell migration to the injuried sites. ICAM-1 does not show a static level of expression, but is up-regulated or down-regulated depending on conditions in the microenvironment [1, 2].

Some studies have suggested that endothelial ICAM-1 expression could increase in response to an unapparent chronic *Chlamydophila* infection, and the soluble form of the adhesion molecule may be secreted in the blood from endothelial cells [13]. Anyway, the antibodies detected by ELISA used in the present study are against the outer membrane complex of *Chlamydophila*, which is released from infected monocytes and macrophages. Accordingly, *C. psittaci* positivity indicates persistent *Chlamydia psittaci* infection of monocytes and macrophages in the blood. Since macrophages could be another source of soluble adhesion molecules, the increased circulating level of sICAM-1 in the *Chlamydia psittaci* seropositive group may not have originated from the endothelium but from circulating monocytes/macrophages chronically activated by the bacterium [2].

Another potential mechanism linking the strong association between *C. psittaci* infection and the high plasmatic level of sICAM-1, is that the tissue lesions modulate the expression of ciculating inflammatory mediators, such as cytokines, ICAM-1 and TNF-alfa. TNF-alfa, in particular, increases the recruitment of inflammatory cells in the course of *C. psittaci* infection, expecially modulating the upregulation of ICAM-1 on endothelial cells; this effect may lead to the development of a chronic infection [3].

To the best of our knowledge, the present study is the first report about the elevated levels of sICAM-1 in seropositive subjects for *C. psittaci* exposed to the occupational risk. Therefore, it is possible to hypothesize a sICAM-1 measurement for use as a tool to verify the development of the *C. psittaci* chronic infection in an occupational setting. However, this hypothesis is merely speculative and the association between high sICAM-1 serum levels and *C. psittaci* seropositivity needs more investigation.

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