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GENERAL GYNECOLOGY

Influence of a mannose-binding lectin gene polymorphism and exposure to *Chlamydia trachomatis* on fallopian tube obstruction in Brazilian woman

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Abstract

Purpose Factors influencing fallopian tube occlusion in women with a lower genital tract infection remain incompletely elucidated. We evaluated whether a polymorphism in the mannose-binding lectin (MBL) gene at codon 54 influences the occurrence of fallopian tube blockage in relation to exposure to *Chlamydia trachomatis*.

Methods In a case–control study at The Hospital das Clínicas, University of São Paulo, Brazil, 75 women with hysterosalpingography-documented tubal occlusion and 75 women with patent fallopian tubes were analyzed for detection of single-nucleotide polymorphism in codon 54 of the MBL gene and for IgG anti-*C. trachomatis* antibodies in their sera. Both groups were matched for age, race, and sexual variables.

Results Prior exposure to *C. trachomatis*, as evidenced by the presence of IgG antibodies, was comparable in both groups. Detection of the polymorphic MBL allele was more prevalent in women with blocked tubes ($p < 0.01$), regardless of whether or not there was evidence of prior chlamydial exposure.

Conclusion The level of MBL-related innate immunity influences the consequences of infection by *C. trachomatis* or other microbes.

Keywords *Chlamydia trachomatis* · Fallopian tube occlusion · Genetic polymorphism · Innate immunity · Mannose-binding lectin

Introduction

Tubal obstruction is estimated to occur in 25–35% of women who have difficultly becoming pregnant [1–3]. The etiology is varied, but pelvic inflammatory disease is identified in > 50% of the cases [1]. Other factors that may lead to tubal blockage include endometriosis, prior pelvic surgery, and genital tuberculosis [4, 5].

Chlamydia trachomatis (CT) is a Gram-negative obligate intracellular bacterium that infects epithelial cells in the eye and the reproductive tract [6]. It is the most common bacterial sexually transmitted infection worldwide. In women, genital CT infection may cause cervicitis, urethritis, endometritis and salpingitis. Recurrent infection or a failure to completely eradicate a current infection is identifiable risk factors for the development of associated sequelae, such as chronic pelvic pain, ectopic pregnancy and tubal factor infertility [7, 8]. Infection with CT has been reported to account for about 40–50% of pelvic inflammatory disease cases and associated tubal occlusion [9, 10]. The various factors associated with CT evasion of the host's immune response, its migration from the lower to the upper genital tract, and its ability to persist and cause disease in the fallopian tubes has not been fully elucidated [11].

Mannose-binding lectin (MBL) is a component of the innate immune system active in the defense against infection, including CT. It binds to carbohydrate residues on

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microbial surfaces and kills the invading cells either by complement-associated lysis or promotion of their opsonization by phagocytic cells [12]. The gene (*MBL2*) coding for MBL is polymorphic, with six known single-nucleotide variations in its DNA sequence [13]. Codon 54 is the most common polymorphic site in White populations. The substitution of guanine for adenine at this site results in production of an unstable MBL protein that is rapidly degraded in the circulation [11, 13, 14]. The normal adenine-containing allele is referred to as the A allele while the guanine-containing variant is referred to as B allele [15].

The MBL codon 54 polymorphism has been shown to increase susceptibility to numerous infectious diseases. There is a higher frequency of the MBL variant allele in women with recurrent vulvovaginal candidiasis (RVVC) [16, 17]. The MBL B allele variant was shown to occur at a significantly higher frequency in women in the United States with vulvar vestibulitis [18]. An association between this polymorphism and laparoscopy-proven fallopian tube occlusion was also reported [11].

We now report on our evaluation of Brazilian women for the codon 54 MBL gene polymorphism and its association with laparoscopy-proven fallopian tube occlusion and evidence of a prior CT infection.

Materials and methods

The subjects in this case–control study were 75 women with occluded fallopian tubes and 75 women with patent tubes seen in the Department of Immunology, Genetics and Infections of Reproductive Care, the Center for Human Reproduction and the Department of Laparoscopy in the Gynecological Clinic Division of Hospital das Clínicas of the Faculty of Medicine of the University of São Paulo. Tubal obstruction was determined by hysterosalpingography and considered abnormal when occlusion of one or both of the uterine tubes was identified. Patients with adherent tubes, which suggested endometriosis or pelvic adhesions, were excluded from the study. The control group was composed of women who were undergoing a tubal ligation for fertility control and who had no evidence of morphological alterations of their tubes at the time of surgery. Tubal patency was confirmed by chromotube. Exclusion criteria included the absence of sexual activity, pregnancy, a diagnosis of malignancy or autoimmune diseases, treatment with antibiotics in the prior 3 months or inability to provide informed consent. The project was approved by the Ethics Committee of the Department of Obstetrics and Gynecology of the Faculty of Medicine of the University of São Paulo, the Ethics Committee for Analysis of Research Projects (CAPPesp) and the National Research Ethics Committee (CONEP). All subjects provided written informed consent. All human investigations were carried out in accordance with The Code of Ethics of the Declaration of Helsinki.

All subjects in both groups answered a questionnaire that included epidemiological data, clinical history, pathological antecedents and gynecological and obstetric history including risk factors for sexually transmitted diseases. Blood was collected from a peripheral vein and following clot formation the serum fraction was collected by centrifugation and stored at − 80 °C until tested. The presence of IgG antibodies to CT was determined with a commercially available ELISA kit (Medac GmbH, Hamburg, Germany). The cutoff value indicating a positive test was as determined by the manufacturer.

The analysis for detection of the codon 54 MBL polymorphism was as previously described [16]. Briefly, DNA was extracted from cells collected from the inside of the cheek (buccal cells) by incubation in a detergent-protease buffer and subjected to gene amplification in the presence of primer-pairs specific for the codon 54 MBL polymorphic region. The resulting amplicons were then treated with Ban I endonuclease that cleaved the wild type but not the variant gene sequences and the sizes of the final products were determined by gel electrophoresis on 2% agarose gels.

Genotype and allele frequencies were determined by direct counting and then divided by the number of chromosomes to obtain allele frequency and by the number of women to obtain genotype frequency. Associations between MBL genotype or alleles and tubal status were analyzed by the Chi square test or Fisher's exact test as appropriate. Goodness of fit to Hardy–Weinberg equilibrium was determined by comparing the expected genotype frequencies with the observed values, using the Chi square test. Associations between demographic and historical variables and tubal status were analyzed by the Chi square test with Yates correction, Fisher's exact test or Mann–Whitney test. A p value < 0.05 was considered significant.

Results

Demographic characteristics and clinical history of both groups of subjects are shown in Table 1. The two groups were comparable in age, race, marital status, age at first sexual intercourse, number of lifetime sexual partners, previous genital tract infections or abdominal surgeries and the number of abortions/miscarriages and preterm births. As expected, the women with patent fallopian tubes had a higher number of pregnancies and births ($p < 0.01$). Women with tubal obstruction reported a greater use of hormonal contraceptives ($p = 0.04$).

Antibodies to CT were detected in a comparable number of women in both groups, 17 (22.7%) in those with blocked tubes and 18 (24.0%) in women with patent tubes.

Table 1 Characteristics of study population

Variable	Blocked tubes	Patent tubes	*p* value
Age in years (SD)	36.4 (5.3)	37.2 (6.2)	NS
Race			NS
White	57.3%	45.3%	
Mixed	30.7%	44.0%	
Black	12.0%	10.7%	
Marital status			NS
Married/civil union	92.0%	86.7%	
Single	5.4%	9.3%	
Divorced	1.3%	2.7%	
Widowed	1.3%	1.3%	
Age at first intercourse			NS
≤15	24.0%	33.3%	
>15	76.0%	66.6%	
No. sexual partners			NS
≤3	64.0%	69.3%	
>3	36.0%	30.7%	
No. pregnancies			<0.01
≤2	88.0%	53.3%	
>2	12.0%	46.7%	
No. births			<0.01
≤2	96.0%	60.0%	
>2	4.0%	40.0%	
No. abortions			NS
≤1	90.7%	92.0%	
>1	9.3%	8.0%	
No. preterm births			NS
≤1	97.3%	96.0%	
>1	2.7%	4.0%	
Use of birth control pills	70.7%	53.3%	0.04
Prior infections	64.0%	61.3%	NS
Prior abdominal surgery	41.3%	29.3%	NS

There were no associations between CT antibody status and any of the demographic variables or sexual antecedents (data not shown).

The distribution of MBL genotypes and alleles in the two groups of women is shown in Table 2. The homozygous A,A genotype was present in 84.0% of women with patent tubes as opposed to 58.7% of those with blocked tubes ($p<0.01$). Conversely, the heterozygous A,B genotype was detected in 36.0% of those with blocked tubes and 16.0% of women whose tubes were open ($p<0.01$). The homozygous B,B genotype was present only in four women with blocked tubes. The allele B frequency was 23.3% in women with occluded tubes vs. 8.0% in the controls ($p<0.01$).

The MBL genotype distribution in the control group was in Hardy–Weinberg equilibrium, but this did not occur in the group with tubal obstruction.

Table 2 Associations between the MBL gene polymorphism and fallopian tube status

Genotype	Allele	Tubal status		*p* value
		Occluded	Patent	
		N=75	*N*=75	
A,A		44 (58.7%)	63 (84.0%)	<0.01
A,B		27 (36.0%)	12 (16.0%)	<0.01
B,B		4 (5.3%)	0	
	A	115 (76.7%)	138 (92.0%)	
	B	35 (23.3%)	12 (8.0%	<0.01

Table 3 illustrates associations between MBL genotypes and alleles and CT serology in women with occluded or patent fallopian tubes. Only in the sub-group of women who had no evidence of being infected with CT was carriage with the variant MBL genotype ($p=0.04$) and allele ($p<0.01$) associated with the occurrence of blocked tubes.

Discussion

In the present study carriage of the MBL variant allele, coding for an unstable protein with reduced antimicrobial activity, was highly associated with the presence of occluded fallopian tubes. This confirmed results of a previous similar study performed in Hungarian women [11]. Since the most common cause of tubal blockage is an upper genital tract infection these findings highlight the role of MBL in protection against a genital tract infection reaching the fallopian tubes and causing an occlusion. Furthermore, the lack of an association between IgG antibodies to CT and the MBL genotype implies that MBL is ineffective in preventing the initial acquisition of a chlamydial

Table 3 Association between MBL gene polymorphism and *C. trachomatis* serology in women with occluded or patent fallopian tubes

Genotype or allele	CT serology	No. (%) positive		*p* value
		Occluded	Patent	
		N=75	*N*=75	
A,A	Positive	10 (13.3)	15 (20.0)	NS
A,B	Positive	7 (9.3%)	3 (4.0%)	NS
A,A	Negative	34 (45.3%)	48 (64.0%)	0.03
A,B	Negative	20 (26.7%)	9 (12.0%)	0.04
B,B	Negative	4 (5.3)	0	NS
Allele B	Positive	7 (9.3%)	3 (4.0%)	NS
	Negative	28 (37.3%)	9 (12.0%)	<0.01

The MBL genotypes and alleles were determined by gene amplification, endonuclease digestion and gel electrophoresis. *C. trachomatis* antibody status was determined by ELISA

infection but is effective in impeding its persistence and ability to infect the fallopian tubes.

Interestingly, of the 115 women with no evidence of a previous CT infection about 50% were found to have a tubal obstruction. In this group also, tubal occlusion was highly associated with carriage of the variant MBL genotype and allele. This strongly suggests that bacteria other than CT were responsible for development of tubal occlusion in our population and that the causative microbes are sensitive to destruction by MBL.

Limitations of the present study need to be stated. The MBL genotype distribution in the control group, but not the group with tubal occlusion, was in Hardy–Weinberg equilibrium. This divergence parallels results of a prior study [11] and, although further investigation is needed, suggests that the altered distribution may contribute to an elevated risk of pathology. At the time of analysis of tubal status women in our study were not tested for CT or other pathogens in their upper or lower genital tract by culture or gene amplification. In the absence of such data we cannot comment on the relationship between MBL genotype and the presence of any microorganism in each subject. The MBL concentration in the vagina or tubal fluid also was not measured and so, although the association between MBL genotype and MBL protein concentration is established[14] we cannot define the MBL level in each subject.

It is interesting to suggest that women who are experiencing infertility might benefit from having their MBL genotype determined or, alternatively, to measure the concentration of MBL protein in their sera or vaginal secretions. Such information might help establish a probable contributing factor to their difficulty in conceiving, i.e., increased susceptibility to a persistent genital tract infection. Increased attention to more extensive bacterial testing may be warranted in these women and appropriate treatment rendered if a potential pathogen is recognized. In addition, knowledge of their MBL status in young women with a recent chlamydial genital tract infection might result in a more individualized treatment regimen to effectively prevent its persistence.

In summary, carriage of the variant MBL codon 54 genotype is associated with fallopian tube occlusion. Expression of the wild-type MBL protein might not prevent acquisition of an initial chlamydial or other infection but, instead, may reduce the probability that a lower genital tract infection will enter the upper genital tract and damage the fallopian tubes.

Author contribution JGV, SCR, IML: trial conception and design, wrote original draft. EIF, RR, JGV, CCO: patient recruitment, sample collection, initial data analysis. IML, SSW: final data analysis and interpretation, wrote final version of manuscript. IML, ECB: study supervision.

Compliance with ethical standards

Conflict of Interest None of the authors report any conflict of interest.

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