

Evaluation of Indirect Immunofluorescence Assay for Diagnosis of *Listeria monocytogenes* in Abortion

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ABSTRACT

Conventional methods for detection of *Listeria monocytogenes* are laborious and time-consuming. Tests that are based on indirect immunofluorescence method (IF), are easy and fast. The aim of this study was the comparison of culturing of amniotic fluid with the indirect IF of serum antibody in abortion and preterm labor. In this study 518 aborted mothers or preterm labor were included. Exclusion criteria were the patients who had been suspicious to non-infectious etiology. The blood and amniotic fluid was obtained in sterile way as specimens. The blood specimen used for indirect IF test and amniotic fluid for microbial culturing. Five hundred and twelve patients completed the inclusion criteria. The age of mothers was between 19 to 42 years with the mean age of 29.83. Twenty-nine patients (5.66%) had preterm labor and 483 (94.33%) patients were aborted. In indirect IF, 6 patients (1.17%) revealed titer of 1:400 and greater for *L. monocytogenes*. In culturing method, in 77 cases (15.03 %) amniotic fluids showed positive results. From these, 5 (0.97%) confirmed for *L. monocytogenes* but 6 cases were positive in IF. In the comparison with gold standard method of culture method, the indirect IF had 100% sensitivity but had 87.8% specificity. Indirect IF should be confirmed by culturing of infected specimens. Determining of anti-listerial antibody in pregnant women and selection of listeriosis for antimicrobial treatment may prevent the spontaneous abortion, still birth and preterm labor. Therefore, we suggest monitoring of *L.monocytogenes* seropositivity in pregnant women with high risk of threatened abortion, and also microbiological assessment of symptomatic women for detection of *L.monocytogenes* and insidious infection.

Key words: *Listeria monocytogenes*, Abortion, Preterm labor, Indirect IF; Culture method.

Introduction

L. monocytogenes is gram-positive bacterium with widespread distribution that causes food-borne infections. Immunosuppression is a promoting factor for severe forms of listeriosis. Pregnancy-associated immunodeficiency makes approximately 20-fold higher risk of infection compared with otherwise healthy adults. These pregnant mothers constitute

60% of all cases of listeriosis [1]. The interaction of InlA–E-cadherin plays a key role in crossing of the placenta barrier in humans and involving the fetus [2]. Preterm labor, amnionitis, spontaneous abortion, stillbirth, and early-onset neonatal sepsis syndrome are the adverse outcomes [3]. The common presentations in pregnancy are preterm labor, decreased fetal activity or fetal death, with an influenza-like illness and meconium-stained liquor, maculopapular rash and hepatosplenomegaly in a

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premature infant [4]. Detection of *L. monocytogenes* is generally performed in a two-step cultural enrichment process and takes on average 5-6 days until the biochemical identification of a *L. monocytogenes* suspicious colony is completed so these conventional methods for detection and identification of *L. monocytogenes* are laborious and time-consuming [5]. Although new methods have been introduced (especially gene-based methods) including the immunoassays [6], nucleic acid hybridization [7], nucleic acid amplification [5, 8-10], lux phage assay [11,12] and combined cultural-molecular methods [13] there is still a real need for detecting optimum detection method [6]. Immunofluorescence microscopy is a very sensitive serological test which harnesses both the power of antibodies to bind to targets along with the use of the fluorescence microscope to visualize the structures to which they bind. Antibody binding is visualized by the fluorescent emission from a marker molecule bound to the antibody. The technique is of particular use in bacteriology and pathology where the location and morphology of the bacterial cells can be viewed along with the location of the fluorescently labeled antibodies. The present report describes the comparison of a conventional cultural methodology of amniotic fluid with the indirect IF of serum antibody in aborted and preterm labored mothers.

Material and methods

This is an analytical study for evaluation of laboratory tests. The Ethics Committee of Azad Tehran University of Medical Sciences approved the study protocol. In this study, 518 pregnant mothers who aborted their fetus or had preterm labor were included. Inclusion criteria were women who had been suspected to infectious etiologies for their preterm labor or abortion. Exclusion criteria were pregnant women with the exact non-infectious etiologies for their preterm labor or abortion such as endocrine dysfunctions, anatomical abnormalities, auto immune diseases, eclampsy, heart and liver diseases and the moles. Six patients were excluded from our study and finally 512 patients enrolled. The biographical data such as age, past medical history were collected and then amniotic fluid and blood samples were obtained. All pregnant women who entered the process of preterm labor or abortion were explained for the aim of study and consent-forms were signed by them.

Sampling:

All blood samples were obtained at sterile condition. Five ml of venous blood obtained and was kept in non-heparin tubes. The tubes were

centrifuged at 3,000 rpm for 15 minutes to separate the serum and were kept in Floulist slides at -20°C until tested by indirect IF. Five to 10ml of amniotic fluid was obtained in the sterile way and inoculated to *Listeria* enrichment medium bottles.

Indirect IF testing:

Four different dilutions 1:100, 1:200, 1:400 and 1:800 were made with phosphate buffered saline (PBS) and sampled serums. Then, fixed Floulist slides were removed from refrigerator and were held at room temperature for 15 minutes. Four 10 µl of different dilutions were put in four sites of Floulist slide. At two other wells of slide, 10 µl of positive and negative serum were put and then the slide was kept at 37°C in an incubator. The slides were removed from incubator, rinsed with the PBS (pH = 7.2) and dried at room temperature. The fluorescent conjugated anti-human antibody was applied onto slide and dried at 37°C for 30 minutes. After washing with PBS (pH=2), the slides were dried again. The 90% glycerin was put on the slide and seen under fluorescence microscope with a 50 × objective lens. Titer ≥ 1:400 was determined as a positive titer.

Culturing:

The enrichment *Listeria* culture bottles were kept in refrigerator (4°C) for one week. Then, 0.1 ml of the container transferred onto blood agar plates and other 0.1 ml onto EMB agar. The culture plates were incubated at 37°C for 24-48 hours. The colonies which were grown on blood agar and EMB plates both were identified as gram negative bacilli, but beta hemolytic colonies that were grown only on blood agar, were examined microscopically and tested biochemically.

Result and discussion

Five hundred and twelve patients completed the inclusion criteria. The age of patients was between 19 to 42 years (mean age of 29.83). Twenty-nine women (5.66%) had preterm labor and 483 (94.33%) were aborted fetus. By indirect IF test 6 cases (1.17%) had titer of 1:400 and greater for *L. monocytogenes* antibodies. In culture, 77 (15.03 %) amniotic fluid samples had positive results (Table-1). 5 (0.97%) cases of *L. monocytogenes* grew in culture. The mothers with positive culture results also had high titer for anti-*Listeria* antibodies. Moreover, there was one case that had high titer of anti-*Listeria* antibody, and negative culture result. There are only 5 positive cultures with high titer of anti *Listeria* antibody in indirect IF of mother's serum. The antibiogram results for *L.monocytogenes* showed 3

resistance strains, 2 for ampicillin, penicillin, cotrimoxazole and cefalothine and 1 for ampicillin, penicillin, tetracycline and cefalothine. All above colonies were susceptible to erythromycin and amikacin. Diagnostic accuracy was assessed from the receiver-operating characteristic (ROC) plot. Diagnosis obtained by conventional cultural examination was selected as the gold standard method and in comparison with sensitivity of IF test was calculated as 100% sensitivity and 82.2% specificity.

Table 1: The other bacteria that were detected in culture.

Name of Bacteria	Frequency	Percent (%)
<i>Enterococcus</i>	17	22.07
<i>Staphylococcus epidermidis</i>	14	18.18
Group B <i>Streptococcus</i>	3	3.89
Group D <i>Streptococcus</i>	3	3.89
<i>S. saprophyticus</i>	1	1.29
Diphtheroid	12	15.58
Gonococcus	1	1.29
<i>E. coli</i>	7	9.09
<i>Actinomyces</i>	3	3.89
<i>Candida albicans</i>	8	10.38
<i>Pseudomonas aeruginosa</i>	1	1.29
<i>Proteus</i>	1	1.29
<i>Enterobacter</i>	1	1.29
Total	77	100

Discussion:

Pregnancy induced immunodeficiency promote *L. monocytogenes* infections higher than normal individuals [1]. Many studies designed in molecular way for determining the pathogenesis of the bacteria and the way of placenta crossing. Nowadays, the authors accepted that ligand-receptor interaction allowing a pathogen to specifically cross the placental villous trophoblastic barrier [2]. The ways for detecting of *L. monocytogenes* were in progress. Conventional determining tests are very laborious and time-consuming which are not beneficial for industries and also scientists. Many study designed to evaluate different tests for diagnosis of this bacteria. Immunoassays [6], nucleic acid hybridization [7], nucleic acid amplification [5,8-10], lux phage assay [11,12] and combined cultural-molecular methods [13] are the examples. Nowadays, the detecting tests were designed for prophylaxis and the tests are applied on foods. Regarding this, PCR technology has successfully shortened analysis time and has been widely applied for the detection of foodborne pathogens [14]. Comparable results were also reported by Herman *et al.* [15] utilizing a nested PCR; however, nested PCR is considered not suitable to diagnostic, because it increases the risk of detecting contaminating PCR-products [16]. Similar sensitivity was also obtained with the phenol-chloroform procedure, with or without the additional step of purification using silica beads. Procedures involving the use of organic solvents were also proposed by other authors [17,18].

However, these methods, even though showed good sensitivity, presents the disadvantage of toxic reagents and the manipulation of organic solvents. In contrast, protocols without organic solvents resulted less sensitive [19]. Many studies were designed for detecting *L. monocytogenes* on foods respectively, but our study was designed for detecting the bacteria in vital fluids and we showed that Indirect Immunofluorescence assay test had the acceptable sensitivity and specificity for detecting.

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