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Original Research

Factors contributes to spontaneous abortion caused by *Listeria monocytogenes*, in Tehran, Iran, 2015

B. Pourkaveh[1], M. Ahmadi[2,*], G. Eslami[3], L. Gachkar[4]

[1] International Branch of Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2] Department of Midwifery and Reproductive Health, School of Nursing and Midwifery, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[3] Department of Microbiology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[4] Infectious Diseases and Tropical Medicine Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Abstract: Spontaneous abortion is the loss of a fetus before the 20th week of pregnancy, when occurring naturally without any surgical or pharmaceutical intervention. On the other hand, *Listeria monocytogenes*, as one of the foodborne pathogens, is a causative agent of listeriosis. The transfer of *L. monocytogenes* in pregnant women occurs as self-limited flu-like symptoms which may result in abortion, stillbirth or premature birth of infected infants. The purpose of this study was the identification of *Listeria monocytogenes* risk factors in women with spontaneous abortion admitted to Tehran Province health care centers in 2015. In this cross-sectional study, 317 women were examined for *L. monocytogenes* using Polymerase Chain Reaction (PCR) and the related risk factors. Two questionnaires on "*L. monocytogenes* Probable Risk Factors" and "Socio Economic Factors" were completed. Out of 317 samples of vaginal swabs, 54 (17%) isolates of *L. monocytogenes* were identified. In addition significant differences in terms of age of mother and her husband, mother and the husband's level of education , house prices, place of residence, gestational age of first abortion, gestational age of current abortion, gestational age of second abortion, consumption of unpasteurized dairy products, consumption of feta and soft cheese, consumption of smoked see food products, consumption of processed meat products and half-cooked meat products, consumption of ready-to-eat vegetables, history of contact with domestic animals three month before pregnancy and during pregnancy and consumption of smoked meat products during pregnancy were studied between two groups of patients positive and negative with *L. monocytogens* ($P < 0.001$). Based on the study, the detection of *L. monocytogens* risk factor during pregnancy as well as taking the issue into account while giving information and counseling in pregnancy can be vital to reduce the incidence of this bacterium and subsequently its side effects during pregnancy.

Key words: Abortion, Pregnancy, *Listeria monocytogenes*, Risk Factors, PCR.

Introduction

The *Listeria monocytogenes* as gram-positive and rod shape bacterium is an intracellular and specious of pathogenic agent which capable of surviving in the presence or absence of oxygen (1). This bacteria found in soil, water, decaying vegetation and a variety of raw foods as well as in processed foods and foods made from unpasteurized milk(2). Beside, listeriosis, a serious infection usually caused by eating food contaminated with the bacterium *L. monocytogenes*. This agent is an important pathogen in pregnant women, neonates, elderly individuals, immunocompromised individuals and Patients with cancer. Listeriosis in pregnant women cause mild illness in mothers but can be devastating to the fetus and leading to a clinical syndrome known as granulomatosis infantiseptica and death(3). In pregnancy for prevents maternal rejection of the fetus a generalized suppression of the adaptive immune system, typified by significantly decreased cell-mediated immunity and reduced T helper cell (Th) 1 responsiveness occurs naturally (4). Unfortunately this immunosuppressed state can lead to increasing maternal susceptibility to certain infectious agents (4). In fact the placenta provides a protective niche for the growth of *L. monocytogenes* and it can cross the intestinal, blood-brain, and placental barriers, leading to gastroenteritis, meningoencephalitis, and maternofetal infections, respectively (5). On the other hand *L. monocytogenes* has ability to escape from killing mechanisms of phagocytic host cells and spread to placenta tissue without exposure to antibodies, neutrophils, or antibiotics in the extracellular fluid. Bacterial colonization of placenta trophoblast cells by production of IFN-γ can induce abortion (6). According to Center for Control Diseases (CDC) one in seven (14%) cases of Listeria infection occurs during pregnancy and pregnant women are about 10 times more prone to Listeria infection than the general population (7).

Also it is reported that Pregnant women account for 27% of all listerial infections (8). Due to potentially severe consequences of listeria infection as a cause of spontaneous abortions and infant mortality, it is highly important that obstetricians, midwifes and pregnancy health care providers be familiar with *L. monocytogenes* routs of transmission, prevention and related risk factors. The aim of this study was to identify *L. monocytogenes* contamination risk factors in patient with spontaneous abortion.

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*** Corresponding author:** Mahbobeh Ahmadi, [2] Department of Midwifery and Reproductive Health, School of Nursing and Midwifery, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Email: mah13751381@gmail.com

Materials and Methods

Study design

This is a cross-sectional study which carried out in Tehran, Iran between January to December 2015. In this survey 318 women 18 to 35 years with complication of spontaneous abortion admitted to medical centers of Tehran Province were studied. Objectives of the study were explained to patients and informal consent was obtained. Two questionnaires included items on possible risk factors of *L. monocytogenes* infection and socio demographic characteristics were completed by all women. The questionnaires were given to women during their stay at hospital with the request to answer them on their own convenience. Then in order to isolation of *L. monocytogenes* mucosal swaps of vaginal wall and cervix were obtained from 318 women and Polymerase Chain Reaction Technique (PCR) was performed on all samples. After performing PCR and identify the positive and negative cases exposure to potential risk factors was evaluated with the help of a questionnaires.

Inclusion and exclusion criteria

Inclusion criteria were pregnant women aging between 18 and 35 years with symptoms of spontaneous abortion such as bleeding, spotting and abdominal cramps with gestational age less than 20 weeks, having no history of infertility, having used no methods of induced abortion and no Assisted Reproductive Techniques (ART), nonsmoking and/or using drugs, lack of chronic diseases such as diabetes and hypertension and abdominal trauma. Exclusion criteria were patient's request to be kept out of the survey as well as wrong answers to the questionnaires.

Measurements

The participants completed two questionnaires which were developed by authors. The questionnaire covered several groups of questions like demographics, reproductive history and history of contact with probable risk factors of *L. monocytogenes* or consumption of probable risk factors of *L. monocytogenes* three months before pregnancy and during pregnancy. These risk factors were consumption of unpasteurized dairy products, consumption of feta cheese or soft cheese, consumption of raw, half baked, processed or smoked meat, consumption of raw or ready to eat vegetables, consumption of raw or smoked sea food, contact with soil and contact with domestic's animal. The socio demographic factors were assessed using questions about mother's age, mother's and father's education, mother's and father's job and employment status, area of residence, living space (m^2), family income, having car, family structure, having personal computer and internet access status. Reproductive history was evaluated with items on parity, previous spontaneous miscarriages and contraceptive methods. In total 36 item were included in this analysis. The questionnaires were applied at face-to-face interviews by the researchers. The questionnaires were answered orally by the participants and recorded by the researchers. In order to eliminate the risk of interviewing the same patient participant's name was written on the questionnaire.

L. Monocytogenes isolation and identification

In this cross-sectional study, a total of 317 samples including vaginal wall and cervix swabs were collected by convenience samplings method from patients with spontaneous abortions who had been admitted to educational medical centers of Tehran, Iran during 2015. These specimens were transferred on ice to the Microbiology Research Laboratory of Shahid Beheshti University of Medical Sciences. In order to *L. monocytogenes* isolation and identification DNA extraction was performed by AccuPrepgenomic DNA extraction kit (Bioneer Co., Korea). The vaginal swab specimens with TSBYE were incubated at 4°C after one week; then 20 µL of proteinase K and 200 µL of binding buffer were added to 200 µL of samples. Samples mixing were done and were incubated at 60°C for 20 minutes, Also 100 µL of isopropanol was added to the mixtures. Centrifuge at 8000 rpm for one minute was performed on lysates and one and then two washing buffer were added to tubes. After finishing centrifuge 200 µL of the elution buffers were added. The final mixture was centrifuged again at 8000 rpm for one minute. The eluted genomic DNA was stored at -20°C for further analyses.

The PCR was standardized for detecting two virulence associated genes of *L. monocytogenes*, namely *plcA*, *hlyA*, using primer pairs of 5'-ATCAGT GAAGGGAAAATGCAAGAAG-3' and 5'-TTGTATAAGCAATGGGAACTCCTGG-3' specific for *hlyA* and 5'-ATGTTAAGTTGAGTACGAATTGCTC-3' and 5'-TACGATGAGCTATAACGGAGACATG-3'specific for *plcA*. The specificity of the standardized PCR was tested by screening the standard strain of *L. monocytogenes* (ATCC 7644). PCR was performed in a final volume of 25 µL, containing 12 µL PCR master mix, 8 vµL double distilled water (DDW), 1 µL (each) primer targeting the *plcA* or *hlyA* gene and 3 µL of the template DNA. The amplifications were carried out in master cycler (Eppendorf Co., Germany) using the following program for *hlyA*: 95°C for one minute, followed by 40 cycles (95°C for 30 seconds, 59 °C for 35 seconds, and 72°C for 30 seconds) and 72°C for five minutes; the amplification program for *plcA* was 95°C for one minute, followed by 40 cycles (95°C for 30 seconds,51°C for 35 seconds, and 72°C for 30 seconds) and 72°C for five minutes. The PCR products bands were analyzed by electrophoresis in a 1.5% agarose gel at 95 V for 45 minutes in Tris-Borate-ethylenediaminetetraacetic acid (TBE) 1X containing ethidium bromide under UV irradiation. The sequencing method was performed by Bioneer Company (Korea) and then the sequences were analyzed with Chromas 1.45 software and BLAST in NCBI.

Statistical analysis

Statistical procedures were conducted using SPSS for Windows 17.0 software package. Means, median, range and standard deviation were calculated for continuous variables. Frequencies and percentages were calculated for categorical variables. Associations between categorical variables were assessed using the chi-squared test or Fischer's exact test as appropriate. Associations between continuous and categorical variables were assessed using Student's t-test as appropriate. $P < 0.05$ was considered significant.

Ethical considerations

The original study was conducted with approval of Shahid Beheshti University of Medical Science Research Ethics Committee (IR.SBMU.IASB.REC). Signed informed consent was obtained from every participant.

Results

A total of 318 women with spontaneous abortion complication, admitted to health care centers of Tehran Province from February 2015 to December 2015 - participated in the study. Due to failure in correctly answering the questions from a questionnaire data form, one of the participants was excluded from the study after two months from the beginning of the survey. The median age of the participants was 26.5 ± 3.9 (ranges between18-35 years of age). All the participants were married (100%), 48.8% completed high school and 53.9% of the participant's husbands had bachelor degree. The majority of the participants were housewives (84.8%) and 3.5% of the husbands were self-employed. 69.4% of them had complete abortion and withdrawal method was the most prevalent contraceptive method among them. The most commonly reported symptoms among the patients were vaginal discharge (100%) and lower abdominal pain (96.3%). PCR method was used in the current study for detection of *L. monocytogenes* and among 317 samples from spontaneous abortions 54 specimens (17%) were positive with *L. monocytogenes* "Figure 1", "Figure 2". All the infected participants were informed of the results and requested to come to Shahid Beheshti University of Medical Science Clinics where relevant treatments were prescribed. The highest rate of infection with *L. monocytogenes* was observed in 18-23 age groups. 66.7% of the infected patients had high school education and 64.8% of their husbands completed high school as well. Given education level, the significant difference was observed between both the groups with infection and no infection ($P < 0.001$). 64.8% of the participants infected with *L. monocytogenes* were housewives and 76% of their husbands were self-employed. 51.9% of the infected patients were tenant and their houses were minimum area of 60 square meters and more. Also, 57.4% of the infected were living in cities. As a result, given the place of living significant difference was noticed between the infected and non-infected groups ($P < 0.001$). Differences in socio-economic profile of both the infected and non-infected participants are summarized in Table 1.

No significant difference was distinguished in 85.2% of the infected participants, used natural contraceptive methods and 72.2% who had complete abortion ($P > 0.001$). In addition, clinical symptoms of vaginal discharge and lower abdominal pain were not associated with *L. monocytogenes* infection. Moreover, parity, abortion age and gestation age were not associated with *L. monocytogenes* infection ($P > 0.001$). Obstetrics characteristics of both groups are summarized in Tables 2 and 3.

In invariable analysis, food exposures were significantly associated with *L. monocytogenes* infection ($P < 0.001$): history of unpasteurized dairy product consumption three months before pregnancy and during pregnancy, consumption of feta or soft three months before pregnancy and during pregnancy, consumption of semi-cooked meat three months before pregnancy and during pregnancy, consumption of smoked meat three months before pregnancy and during pregnancy, consumption of processed food three months before pregnancy and during pregnancy, consumption of smoked seafood three months before pregnancy and during pregnancy, consumption of ready-to-eat vegetables during pregnancy (Table 4). Furthermore, having contact with domestic animals as well as soil three months before pregnancy and during pregnancy were significantly associated with *L. monocytogenes* infection ($P < 0.001$) (Table 4).

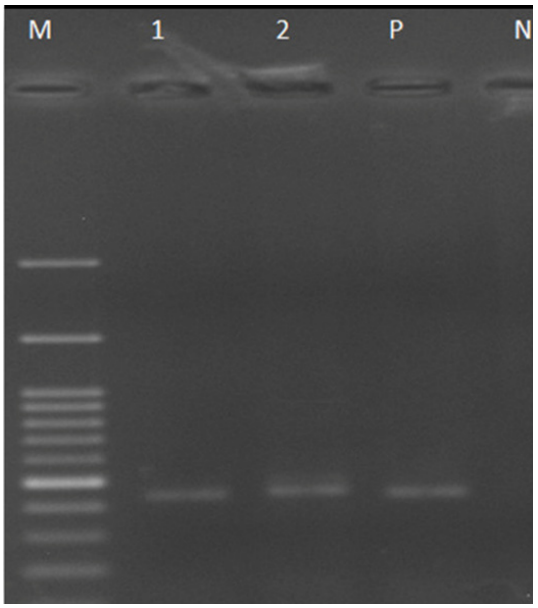

Figure 1. Polymerase Chain Reaction Products of *hlyA* gene (451 bp) on 1.5% Agarose Gel Stained With Ethidium Bromide.

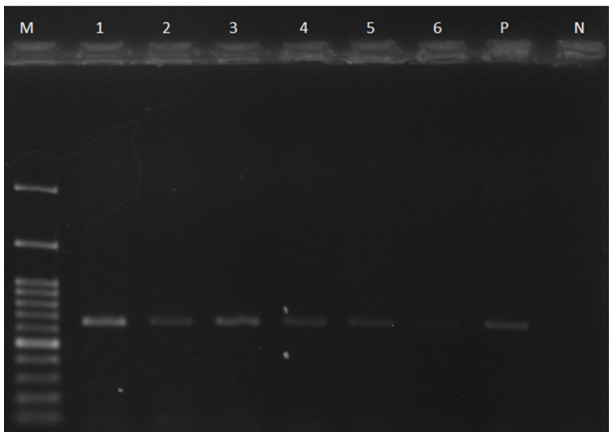

Figure 2. Polymerase Chain Reaction Products of *plcA* Gene (659 bp) on 1.5% Agarose Gel Stained With Ethidium Bromide.

Discussion

Infection with *L. monocytogenes* is prevalent worldwide, since it has been isolated from many sources such as a variety of foods, vegetables, animals and soil (1). The prevalence of *L. monocytogenes* (17%) found in this study is comparable with that of which has been reported in previous studies carried out on women in Iran. Shayan *et al*. reported 36% isolates of *L. monocytogenes* in 100 vaginal sample in Iran by PCR method (9). In research study, Jahangirsisakht *et al*. collected

Table 1. Distribution of Socio economic Factors in patient with spontaneous abortion in two groups of with and without *L. monocytogenes* infection admitted to health care center of Tehran Province, Iran, 2015.

Variable	With *L . monocytogenes* infection n=54	Without *L. monocytogenes* infection n=263	P value
Age			
18-23	19(35.1%)	25(9.5%)	P<0.001
24-28	18(33.3%)	170(64.6%)	P<0.001
29-33	11(20.3%)	40(15%)	P<0.001
34-35	6(11.1%)	28(10.4%)	P<0.001
Total	54(100%)	263(100)	-
Mother Education level			
Middle school	3(5.6%)	0(0%)	P<0.001
High school	36(66.7%)	119(45.2%)	P<0.001
Associate Degree	1(1.9%)	8(3%)	P<0.001
Bachelor	11(20.4%)	123(46.8%)	P<0.001
Master	0(0%)	9(3.4%)	P<0.001
Phd	3(5.6)	4(1.5%)	P<0.001
Total	54(100%)	263(100)	-
Husband education level			
Middle school	1(1.9%)	4(1.5%)	P<0.001
High school	35(64.8%)	80(30.4%)	P<0.001
Associate Degree	0(0%)	2(0.8%)	P>0.001
Bachelor	13(24.1%)	158(60.1%)	P<0.001
Master	1(1.9%)	15(5.7%)	P<0.001
PhD	14(7.4%)	4(1.5%)	P<0.001
Total	54(100%)	263(100)	-
Mother Job			
Housewife	19(35.1%)	83(31.5%)	P>0.001
Employment	35	180(68.4%)	P>0.001
Total	54(100%)	263(100)	-
Husband Job			
Unemployed	0(0%)	2(8%)	P>0.001
Laborer	7(13%)	25(9.5%)	P>0.001
Self-employed	41(76%)	210(79.8%)	P>0.001
Employed	6(11.1%)	26(9.9%)	P>0.001
Total	54(100%)	263(100)	-
Having House			
Owner	28(51.9%)	124(47.3%)	P>0.001
Tenant	24(44.4%)	128(48.9%)	P>0.001
Living with relatives	2(3.7%)	10(3.8%)	P>0.001
Total	54(100%)	263(100)	-
Floor area per person			
25 to 60 square meters**.**	13(24.1%)	89(33.8%)	P>0.001
More than 60 square meters	41(75.9%)	174(68.7%)	P>0.001
Total	54(100%)	263(100)	-
Place of living			
City	22(40.7%)	181(68.8%)	P<0.001
Village	1(1.9%)	0(0%)	P<0.001
Suburb	31(57.4%)	113(35.6%)	P<0.001
Total	54(100%)	263(100)	-
Having personal Car	24(44.4%)	170(64.6%)	P<0.001
Having Personal Computer	4(7.4%)	59(22.4%)	P>0.001
Having Internet Accesses	22(40.7%)	213(81%)	P<0.001

Table 2. Distribution of Contraceptive method among patient with spontaneous abortion in two groups of with and without *L. monocytogenes* infection admitted to health care center of Tehran Province, Iran, 2015.

Variable	With *L. monocytogenes* infection n=54	Without *L. monocytogenes* infection n=263	P value
Condom	5(9.35)	35(13.3%)	P>0.001
OCP	2(3.7%)	22(8.4%)	P>0.001
IUD	0(0%)	3(1.1%)	P>0.001
Contraceptive Injection (one month interval)	1(1.9%)	12(4.6%)	P>0.001
Contraceptive Injection (Three month interval)	0(0%)	3(1.1%)	P>0.001
Withdrawal	46(85.2%)	188(71.5%)	P>0.001
Total	54(100%)	263(100)	

Table 3. Distribution of obstetrics characterization among patient with spontaneous abortion in two groups of with and without *L. monocytogenes* infection admitted to health care center of Tehran Province, Iran, 2015.

variable	With *L monocytogenes* infection n=54	Without *L. monocytogenes* infection n=263	P value
Gravidity	1.6	1.5	P>0.001
Parity	0.5	0.4	P>0.001
Abortion	1	1.1	P>0.001
Gestation age of current abortion(per week)	8.6	10.1	P<0.001
Gestation age of First abortion(per week)	8.6	9.9	P<0.001
Gestation age of second abortion(per week)	10.2	11	P>0.001
Gestation age of third abortion(per week)	0	13	P>0.001

Table 4. Distribution of *L. monocytogenes* risk factors *among* patient with spontaneous abortion in two groups of with and without *L. monocytogenes* infection admitted to health care center of Tehran Province, Iran, 2015.

Variable	With *L . monocytogenes* infection n=54	Without *L. monocytogenes* infection n=263	P value
Consumption of unpasteurized dairy products			
Three months before pregnancy	42(78.8%)	2(0.8%)	P<0.001
During pregnancy	37(68.5%)	2(0.8%)	P<0.001
Consumption of soft cheese			
Three months before pregnancy	45(83.3%)	4(1.5%)	P<0.001
During pregnancy	40(74.1%)	11(4.2%)	P<0.001
Consumption of raw meat			
Three months before pregnancy	2(3.7%)	3(1.1%)	P>0.001
During pregnancy	1(1.9%)	0(0%)	P>0.001
Consumption of semi cooked meat			
Three months before pregnancy	28(51.9%)	6(2.3%)	P<0.001
During pregnancy	25(46.3%)	5(1.9%)	P<0.001
Consumption of smoked meat			
Three months before pregnancy	1(1.9%)	3(1.1%)	P<0.001
During pregnancy	18(33.3%)	4(1.59%)	P<0.001
Consumption of processed meat			
Three months before pregnancy	16(29.6%)	8(21%)	P<0.001
During pregnancy	27(50%)	1(0.4%)	P<0.001
Consumption of smoked sea food			
Three months before pregnancy	15(27.8%)	3(1.1%)	P<0.001
During pregnancy	12(22.2%)	22(8.4%)	P<0.001
Consumption of ready to eat vegetables			
Three months before pregnancy	6(11.1%)	5(1.9%)	P>0.001
During pregnancy	17(31.5%)	5(1.9%)	P<0.001
History of contact with domestic animal			
Three months before pregnancy	27(50%)	7(2.7%)	P<0.001
During pregnancy	21(46.2%)	5(1.9%)	P<0.001
History of contact with soil			
Three months before pregnancy	14(25.9%)	11(4.2%)	P<0.001
During pregnancy	21(46.2%)	5(1.9%)	P<0.001

311 samples of urine, blood, placenta and cervix swab from 107 pregnant women in Iran, *L. monocytogenes hlyA* gene was detected in 11 women (10.28%) (10). In another case-control study in Iran, Tahery *et al*. surveyed 204 in-patient women with and without abortion in relation to antibody against *L. monocytogenes* using Indirect Imnoflorescent method and 7.4%had antibody against *L. monocytogenes*; 5.9% in abortion group and 1.5% in non-abortion group (11). In addition, Soni *et al*. characterized *L. monocytogenes* isolated from pregnant women in a study in India. They surveyed 3700 clinical samples of placenta and vaginal swap by REP-PCR fingerprint analyses, among which a total of 30 isolates (0.81%) [12 (0.80%) from placental bit (1500) and 18 (0.81%) from vaginal swab (2200)] had positive *L. monocytogenes* (12). It can be said that he differences between the obtained prevalence of *L. monocytogenes* in Soni *et al*. study and the present study can be due to the difference in case study; the population of the present study is women with abortion while that of the upper-mentioned study is pregnant women who completed their pregnancy period. Overall, it indicates that the prevalence of *L. monocytogenes* is still significant among female population, which can have adverse consequences for both fetus and mother.

In present study, mean age of the participants who was infected with *L. monocytogenes* was 23. 4 ± 3.2. Tahery *et al*. maintain that the main prevalence of *L. monocytogenes* was observed in age ranges of 41-45 years old and 36.4% of women in this age range were infected by *L. monocytogenes* in Iran (11). Due to the effect of high impact of age above 35 years on occurrence of abortion (13), women older than 35 years were excluded from the present study. Shayan *et al*. claim

that the highest incidence of infection with *L. monocytogenes* was observed in women between 20- 29 years old (9). Although this study was conducted among non-pregnant women, it shows that infection with *L. monocytogenes* is widespread among women in reproductive ages and can have adverse consequences relating to pregnancy.

In another study conducted by Siegman-Igra *et al*. (14) in Israel, 69 pregnant women infected with *L. monocytogenes* with age range of 21-40 years were investigated in order to investigate *L. monocytogenes* in the country and to review worldwide cases, the mean age was 28 years (14). In this study, 161 cases were included during five years; 87 nonperinatal cases and 69 pregnant women were studied. The mean age of nonperinatal cases was 67 years (ranges between 4–91 years old), 64 (74%) of whom had severe immunocompromising conditions. Hence, it seems that reduced immunity either in pregnant women for physiological reasons or in other people due to diseases and/or other reasons has a role to play in development of *L. monocytogenes* infection.

In present study it is gathered that women who are infected with *L. monocytogenes* had lower level of education (high school degree 68.4%) in comparison with non-infected women (bachelor degree 60.1%). It appears, there was significant statistical differences between the two groups ($P < 0.001$). In the study conducted in England, Tam *et al*. declares that individuals who are in poor socio-economic conditions which defined by age at leaving full-time education and housing were more prone to infectious diseases a general practice than community controls were (15). In Tanzania in a cross sectional study carried out on 295 pregnant women in relation to Maternal vaginorectal colonization by Group *B Streptococcus* and *L. monocytogenes*, and its risk, Ernest *et al* factors found that decrease in the level of education tends to result in increased microbial colonization (16). Considering these studies, it seems that level of education has important effect on hygiene practices and prevention from infectious agents such as *L. monocytogenes*.

In the present study, statistically significant relationship was observed between the two groups in terms of living environment ($P < 0.001$); 54.7% of the infected patients were livening in suburbs, while 68.8 % with no infection were living in the cities. The obtained result can be due to the agricultural lands, livestock places and consumption of local dairy product in suburbs of Tehran, increasing probable risk of contact with *L. monocytogenes*. Furthermore, there were statistically significant differences between the two groups in terms of Internet access ($P < 0.001$); 40.7% of whom detected with infection and 81% without infection had Internet access. It seems that in addition to level of education, medical information about the Internet can improve the individual's knowledge to maintain and improve their health. In a study by Huberty *et al*., 293 women - who were currently pregnant or up to one year postpartum – were participated in the USA in order to determine how pregnant women use the Internet for health issues during pregnancy through the information related to physical activity and nutrition. Almost all women (94%) reported using the Internet for pregnancy related information. Women reported using the Internet for general health information about pregnancy were six to ten times more informed on the issue than others. Half of the women used the Internet for information related to physical activity during their pregnancy and some increased their physical activity as a result. Therefore, they could pass their abortion with less hazard (17).

The most prevalent contraceptive method among women with infection of *L. monocytogenes* was withdrawal method 46 individuals (85.2%) and there was no statistical difference in relation with contraceptive method between infected and non-infected women. In Ernest *et al*. study, there was no association in regard to contraceptive method and susceptibility to infectious agents (16). Besides, in the Manual of Prinatal Infection by Vandana Walvekar MJ Jassawalla *L. monocytogenes* is considered as an infection which is not a sexually transmitted disease (18). this study, 78.8% of the patients with *L. monocytogenes* and 0.8% without *L. monocytogenes* infection had the history of unpasteurized dairy products consumption three months prior to their pregnancy ($P < 0.001$). Moreover, 68.8% of the patients with *L. monocytogenes* and 0.8% of them without *monocytogenes* infection had history of unpasteurized dairy products consumption during pregnancy ($P < 0.001$). In the study by Tahery *et al*. (11)50.9% of abortion patients had history of using dairy products (11). According to a study by Linnan *et al*, most of the *L. monocytogenes* dairy-associated outbreaks are due to inadequate pasteurization and unpasteurized dairy products (19). Girard *et al*. from France assert that 20% of pregnant women who were infected with *L. monocytogenes* had history of consumption of unpasteurized dairy products (*20*). Holko *et al*. from Slovenia could detect *L. monocytogenes* by 18 (18%) positive dairy products samples, traditional cultivation method and nested PCR from 100 dairy products samples (21). Likewise, Harakeh *et al*. from Lebanon isolated *L. monocytogenes* by 30 (18%) samples of 164 dairy products by PCR (22). These studies confirm the existence of *L. Monocytogenes* in dairy products resulting in high risk of transmission during pregnancy.

In the current study, 83.3% of positive and 1.5% of negative *L. monocytogenes* patients had consumed soft cheese three months before pregnancy ($P < 0.001$), as well 74.1% of positive and 4.2% of negative *L. monocytogenes* patients had consumed soft cheese during pregnancy ($P < 0.001$). In a study from Jordan by Osaili *et al*., 350 samples of five different types of brined white cheese have been investigated by PCR and *L. monocytogenes* was isolated by 39 (11.1%) samples (23). In another study by Valentina Coroneoin from Italy, 252 samples of Ricotta Salata - which is a traditional ripened and salted whey cheese - have been examined and 87 samples i.e. 17.2% were positive with *L. monocytogenes* (24). It is deemed that cheese is a proper environment for *L. monocytogenes* growth and survival, as a result it becomes a major concern to the public health especially for pregnant women.

In current study, it was found that 29.6% of the patients with *L. monocytogenes* and 3% with no *L. monocytogenes* had history of consumption of processed meat products three months before pregnancy and 50% of whom with *L. monocytogenes* and 0.4% without *L.*

monocytogenes had history of consumption of processed meats products during pregnancy (P < 0.001). In study by Guangyu Wang in China, *L. monocytogenes* has been found by 33 (5.3%) of 628 samples used ready-to-eat meat products (25). Also, Al-Nabulsi *et al*. found 24.4% *L. monocytogenes* prevalence in 270 samples of raw and processed meat products using culture method and PCR (26). It should be mentioned that in the present study there was significant statistical differences in terms of consumption of smoked meat products and semi-cooked meat products between the two infected and non-infected groups (P < 0.001). However given raw and uncooked meat products, no significant difference was found (P > 0.001), which is probably due to Iranian nutrition culture in terms of avoiding the consumption of raw meat by the people.

Similarly, regarding consumption of smoked sea food products significant association was also observed. 27.8% of infected patients three months before pregnancy and 22.2 % of infected patients during pregnancy had history of smoked sea food consumption (P < 0.001). In Girard *et al*. study in France, 33% (165) of the abortion patients and *L. monocytogenes* infection had history of smoked seafood consumption (20).

In a study by González *et al*., 250 samples of fish by ISO 11290-1/A1 and ISO 11290-2/A methodologies were examined and 0% to 66.7% of smoked salmon were *L. monocytogenes* positive depending on the brand (27). In another study by Das *et al* , a total of 324 tropical seafood and fishery environmental samples were screened for *L. monocytogenes* using multiplex PCR and Listeria spp. was detected in 32.3%, 27.1% and 5 % of fresh, frozen and dry fish samples, respectively (28). Although the natural nitch for *L. monocytogenes* is soil, it seems that sea food is also a good and suitable micro environment for growth and survival of this bacteria.

Of 54 patients who were positive with *L. monocytogenes* 17 (31.5%) had history of raw or unwashed vegetables consumption during pregnancy while 5 (1.9%) of 263 non-infected patients consumed these vegetables during pregnancy. Considerable association regarding the use of raw or unwashed vegetables during pregnancy as well as infection with *L. monocytogenes* was observed in the two groups (P < 0.001). In a study by Jamali *et al*. in Iran, 300 samples of ready-made mayonnaise and vegetables salads were examined in order to find *L. monocytogenes.* To this end, the microbial identification was applied by biochemical tests and it was confirmed by duplex PCR. A total of 8.7% of the samples harboured Listeria spp., including 7% *Listeria monocytogenes*, 1% *Listeria innocua* and 0.7% *Listeria welshimeri* (29). According to a study by Roger Stephan, from 26 October 2013 to 23 April 2014, 32 cases of listeriosis infected with *L. monocytogenes* were identified. After one year a food producing company was reported to have *L. monocytogenes* contamination of ready-to-eat salads, which was related to outbreak strain. Henceforth, due to increasing use of ready-to-eat vegetables and salads among people and since there is no reliable source on the correct way of washing methods people and specially pregnant women are at risk of contamination with microbial pathogen (30-32).

In current study, 50% of the patients with positive *L. monocytogenes* three months before pregnancy and 46.2% during pregnancy had history of contact with domestic animals (P < 0.001). In a study by Keelara *et al*., a total of 880 clinical samples comprising blood (n = 215), vaginal swabs (n = 220), fecal swabs (n = 220), placenta (n = 10) and sera (n = 215) from ewes which had abortion and history of the abortion were investigated in order to find *L. monocytogenes.* Twenty three different types of *L. monocytogenes* were isolated, including 15 *L. monocytogenes*, 2 *L. ivanovii* and 6 other listeriae (33). In study conducted by Sarno *et al* in Switzerland, 504 tonsil samples of slaughtered fattening pigs were studied by culture enrichment and 28 samples (5.6 %) were positive with *L. monocytogenes* (34). As mentioned before, based on the current study 54.7% of the infected patients were livening in suburbs where agricultural lands and animal husbandry are mainly located. Therefore, there exists high chance of having contact with soil and domestic animals for pregnant women living in suburbs near Tehran.

It became more evident that contact with soil is more common in patients with *L.monocytogenes* (25.9% three months before pregnancy and 26.4% during pregnancy) (P < 0.001). In a study by Dharmendra Soni *et al*., 200 vegetable samples and respective rhizosphere soils (soil adhering to the root surface) for the presence of *L. monocytogenes* were scrutinised and 20 (10%) of vegetable samples and 10 (5%) from 200 soil samples were positive using ERIC- and REP-PCR approach. This study provides evidence for the prevalence of *L. monocytogenes* in farm samples and soil (35).

It appears that *L. monocytogenes* incidence is high among pregnant women in Tehran Province; therefore pregnant women and their health care providers should be informed about listeriosis during pregnancy. Pregnant women health care providers like midwives and obstetrics should be the credible source of information for pregnant women and provide them with accurate advice about the risks associated with listeriosis. Most cases of listeriosis in pregnancy could be prevented not only by avoiding the consumption of certain foods and contact with certain animals but also by proper food preparation.

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