

Full Length Research Paper

## Assessing *Listeria monocytogenes hly A* gene in pregnant women with spontaneous abortion using PCR method in Yasuj, south west of Iran

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Accepted 10 May, 2013

Listeriosis is a disease which is caused by *Listeria monocytogenes* bacterium that endangers lives of infants, pregnant women, elderly and patients with suppressed immunity system. The aim of this study was to assess the prevalence of *L. monocytogenes* in pregnant women with spontaneously abortion and isolating it using polymerase chain reaction method in Yasuj. In a cross-sectional study, 311 specimens were collected from 107 pregnant women including urine, blood, placenta and cervix swab. Among the specimens, 190 samples were from pregnant women with history of abortion and 120 samples from the cases with normal delivery. The specimens were enriched for a period of 4 weeks at 4°C. The samples were studied for *hly A* genome using polymerase chain reaction method. Among 107 pregnant women, in 11 cases (10.28%), *L. monocytogenes hly A* gene was detected, with the highest frequency in the age group of 26-30 years old. The mean age of participants was 26.7 years and almost 64% of the positive cases belonged to the cervix. The results of this study show that pregnant women should be checked for *L. monocytogenes*. Also, further study on newborns with meningitis for this bacterium is recommended in the region.

**Key words:** *Listeria monocytogenes*, abortion, pregnant women, *hly A*.

### INTRODUCTION

*Listeria monocytogenes* is a pathogenic bacterium which is able to cause listeriosis sporadically. Although the incidence of the disease is low, the case fatality rate is high (Mahdizadeh et al., 2010). In most cases, the bacterium is transmitted by taking contaminated food stuffs (Sedighimoghadam, 2001). This bacterium threatens the lives of infants, elderly and individuals with suppressed immunity system (Ramaswamy et al., 2007). In particular,

pregnant women are at risk of being affected, and their body is susceptible for the growth of this bacterium (Kargar and Ghasemi, 2009). As a result of the infection by the bacterium, pregnant women will face various complications including preterm delivery, spontaneously abortion, stillbirth and neonatal infection which are followed by a high rate of mortality (Low and Donachie 1997; Janakiraman, 2008). The chances of being affected to

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**Table 1.** primer sequence use in this study.

Target	Primer sequence	Product size (bp)
<i>hly A</i>	F.L.M: 5'TGT TAA TGA ACC TAC AAG ACC TTC3'	250
	R.L.M: 5'TAG TTC TAC ATC ACC TGA GAC AGA3'(5)	

this bacterium in pregnant women are 20 times more likely than healthy adults, and they account for 27% of all cases of listeriosis (Ogunmodede et al., 2005). Most people who are infected have few or no symptoms or have symptoms similar to a mild common cold, which is diagnosed by blood culture (Pourjafar et al., 2011).

With respect to important characteristics of this bacterium, which mainly lives intracellular in the immunity system, and its ability to spread inside the body, special attention must be paid to stuffs which are used as food (Mahdizadeh et al., 2010; Rahimi et al., 2011).

Therefore, with respect to the longtime needed to get the results of culture and difficulties arisen from isolation of this bacterium, the aim of this study was to assess the prevalence of *L. monocytogenes* in pregnant women with spontaneously abortion and isolating it using polymerase chain reaction method in Yasuj, a city in south west of Iran.

## MATERIALS AND METHODS

This is a cross-sectional study in which took place in September 2008 to June 2009. 311 specimens were collected from 107 pregnant women with history of abortion, aborting or with normal delivery. The specimens included urine, blood, placenta and cervix swab. In this study, 2 ml of urine sediment, 10 ml of blood, and 25 g of placenta tissue and cervix swab were homogenized in brain heart infusion broth (BHIB) and placed at a 4°C cold enrichment for a period of four weeks.

After 4 weeks, the enriched specimens were analyzed to trace *L. monocytogenes hly A* gene using molecular polymerase chain reaction. During all stages of cold enrichment and performing tests, the *L. monocytogenes* (PTCC: 1163) were used for quality control. The standard protocol of polymerase chain reaction including 25 ul buffer, 1 ul MgCl<sub>2</sub>, 0.25 ul dineucleotide triple phosphate.0.1 ul of Taq polymerase and 0.5 ul of each of forward and reverse primers of *hly A* genome was performed (Table 1).

Also, available cycles included 95°C for 5 min, 32 cycles, 94°C for 40 s, 58°C for 40 s, 72°C for 40 s and at the end 72°C for 5 min. Finally, the obtained product of polymerase chain reaction was studied on agarose gel 2% by electrophoresis. After staining with ethidium bromide, the product was studied using transilluminator ultra violet light. Statistical analysis was carried out with SPSS software and significant level was set at  $p < 0.05$ .

## RESULTS AND DISCUSSION

The mean age of the participants in this study was 26.7 years old. The collected specimens included cervix (34.5%), blood (21.5%), urine (22.5%) and placenta (21.5%). The highest frequency of abortions was seen in the age group of 26-30 years. The level of education of almost 36% of the participants was diploma, 90% of them

were house wives and 65.5% of the subjects had used dairy products in their diet several days before the abortion. In total, from 107 pregnant women in this study, 11 specimens (10.28%) *hly A* genome were isolated (Figure 1).

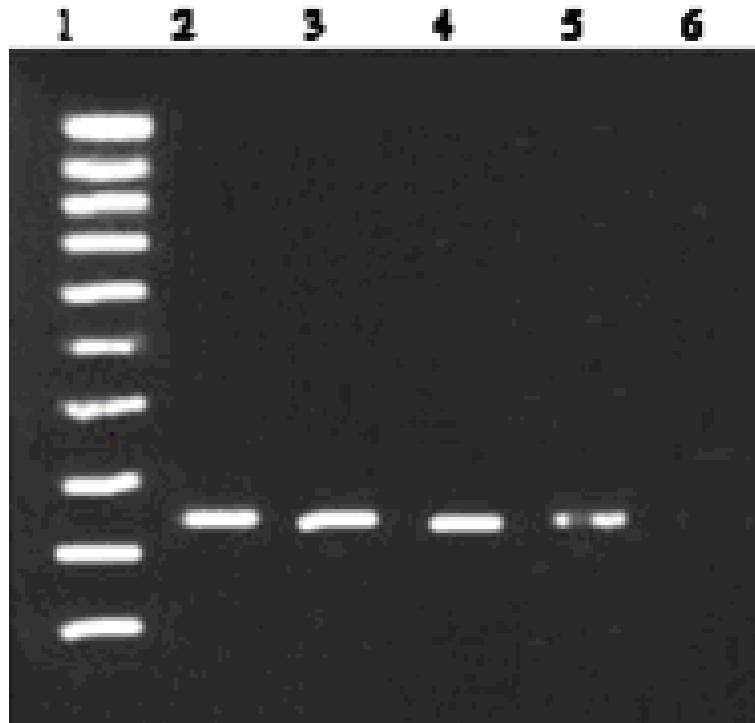
Among positive specimens, 63.6% of the specimen belonged to cervix, 36.4% to urine and 18% to urine and cervix.

No significant correlation was observed between the level of education, using dairy products, contact to live-stock, number of pregnancy and abortion with the *hly A* genome isolation. Positive and negative results of polymerase chain reaction in different specimens are shown in Table 2 based on the number of abortions. The highest positive cases were observed from the specimens of cervix and in the first abortion (Table 2).

*L. monocytogenes* is the bacterium that causes the infection listeriosis. It can grow and reproduce inside the host's cells and is one of the most virulent food-borne pathogens that finally lead to septicemia, meningitis and abortion (Pourjafar et al., 2011). The actual status of listeriosis in pregnancy in Iran is unknown and little information is available about the presence of *L. monocytogenes* in Iran. It also is not a disease which is routinely monitored or reported by health sectors in Iran.

Moreover, there is not any recommendation or study based on presence of listeria *menocytogenes* in Iran (Bakardjiev, 2006). The disease is diagnosed by culture of clinical samples from blood, placenta, stool, vaginal secretion, cerebral spinal fluid. Because of scantiness of the bacteria and difficulty in differential diagnosis with other bacteria, this method is not routinely used in many laboratories (Shayan, 2009). Therefore, the objective of this research was to study the status of pregnancy listeriosis in pregnant women with history of abortion in Yasuj.

More than one third of reported cases of listeriosis during pregnancy have been in the second and third trimester of pregnancy and have led to spontaneous abortion (Stepanovic et al., 2007). Also, the presence of listeria has also been reported in the first trimester of pregnancy. Jamshidi et al. (2009) reported that the clinical symptom of 48% of patients was fever, and in 61% a syndrome similar to common cold was observed (Jamshidi, 2009). However, Moledo et al. (2008) mentioned that most cases of listeriosis in pregnancy have intermediate symptoms or there were no symptoms at all (Moledo et al., 2008). In a study conducted by Jamshidi et al. (2009) in Bandar Abbas, 35.6% in the test group and 17.5% in the control group were seropositive for *L. monocytogenes* and significant correlation was also observed between



**Figure 1.** Electrophoresis of *Listeria monocytogenes hly A* gene with 250 bp product. 1, marker 100 bp; 2, positive control (PTCC;1163); 3,4 and 5, positive sample; 6, negative control.

**Table 2.** positive and negative specimen of *listeria monocytogenes* in polymerase chain reaction method in separation of number of abortion in pregnant women of Yasuj.

Number of abortion	Specimen								Total
	Urine		Cervix		Placenta		Blood		
	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	
Without abortion	23 (7.39)	2 (0.64)	25 (8.03)	0 (0)	25 (8.03)	0 (0)	25 (8.03)	0 (0)	100 (32.15)
First abortion	29 (9.32)	3(0.96)	58 (18.64)	6 (1.92)	32 (10.28)	0 (0)	32 (10.28)	0 (0)	160 (51.44)
Second abortion	10 (3.21)	1(0.32)	12 (3.85)	1 (0.32)	8 (2.57)	0 (0)	8 (2.57)	0 (0)	40 (12.86)
Third abortion	2 (0.64)	0 (0)	5 (1.60)	0(0)	2 (0.64)	0 (0)	2 (0.64)	0 (0)	11 (3.53)
Total	64 (20.57)	6 (1.92)	100 (32.15)	7 (2.25)	67(21.54)	0 (0)	67 (21.54)	0 (0)	311 (100)

the two groups (Jamshidi et al., 2009).

In 2008 Moledo et al. could isolate *L. monocytogenes* from CSF specimen of a seven month old infant (Moledo et al., 2008).

In a research carried out in india by Kaur et al. in 2007 on spontaneous abortion, they isolated 14.8% specimen of listeria and 3.3% *L. monocytogenes*. In the research, they also studied *plcA*, *prf A*, *act A*, *hly A* and *iap* genomes (Kaur et al., 2007).

In the present research, *hly A* genome was also used for study and among 107 pregnant women, 11 cases (10.3%) hemolysin *L. monocytogenes* genome were isolated. Although no significant correlation was observed between infection of this bacterium and abortion, but with

respect to the obtained results and ability of the bacterium to contaminate infants at the time of birth and its ability to cause repeated abortion, it is necessary to include the study of this bacterium as a factor of abortion in the health items.

It seems that research on several different pathogenic genomes in any study will provide better results. Therefore, further research is proposed on infant meningitis in the studied region.

**ACKNOWLEDGEMENTS**

We express our gratitude and thanks to the authorities of Yasuj University of Medical Sciences which provided the

expense of the study by a grant number 6918. We also extend our gratitude and thanks to the laboratory staff and maternity center of Emam Sajjad Hospital (yasuj) for their heartily cooperation.

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