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Detection of *Listeria monocytogenes* in Placenta of Aborted Women

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Abstract

Background *Listeria monocytogenes* (*L. monocytogenes*) is a Gram-positive, facultative intracellular bacterial pathogen that can cause a severe invasive disease (listeriosis), mainly in immunocompromised, elderly individuals, and pregnant women, characterized by sepsis, neonates and miscarriage.

Objective To evaluate the association of listeria monocytogenes in abortion in a group of Iraqi women.

Methods A cross-sectional study was designed and included 250 placenta tissues obtained from aborted women, each placenta sample was divided into two parts each about 50 gm in weight, one stored in 10% formaldehyde solution to use for histopathological study using Hematoxylin and Eosin (H&E) staining procedure, while the second part cut into small pieces about 15 gm and washed and stored in 5 ml normal saline solution 0.85% to use in detection of the presence of the bacterium using conventional bacteriological methods.

Results Out of 425 placenta samples only 250 were diagnosed to be placentitis and only 15 isolates of *L. monocytogenes* were isolated and constitutes 6% of the total number of placentitis. Distribution of placentitis due to listeria and age groups have been shown no statistical significant differences (P = 0.099). Also, there was no statistical significance difference between the percentage of isolated *L. monocytogenes* and the time of gestation in aborted women with placentitis (P value was 0.689), and also no significance in association between number of abortion and isolation of *L. monocytogenes* (P = 0.689).

Conclusion No association between *L. monocytogenes* and recurrent abortion or with time of gestation or the age of the patients.

Keywords *Listeria monocytogenes*, placenta, aborted women

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List of abbreviations: *L. monocytogenes = Listeria monocytogenes*

Introduction

Listeria monocytogenes (*L. monocytogenes*) is a Gram-positive, non-spore-forming, motile, facultative anaerobic, rod-shaped intracellular bacterial pathogen that can cause a severe invasive disease (listeriosis), mainly in immunocompromised, elderly individuals, and pregnant women, characterized by sepsis, meningitis and miscarriage [(1)].

Listeriosis during pregnancy may lead to intrauterine infection, which may result in severe complications like preterm labor, spontaneous abortion, and stillbirth and/or infection of the neonate which may result in high morbidity and mortality rates [(2)].

Despite its clinical importance, a little is known about the molecular and cellular mechanism leading to placento-fetal infection, or the role

of pregnancy in the development of listeriosis. One explanation for the increased susceptibility to listeriosis during pregnancy is the immunological conditions of mammalian reproduction, where the maternal immune system tolerates paternal alloantigens expressed in fetal tissues. Since then, pregnancy has been regarded as a state of immunosuppression; in particular, of the cell-mediated arm of the immune system [3].

A decrease in cell-mediated immunity might explain the increased susceptibility to infection with the bacterial pathogen, *L. monocytogenes* and this is the reason for increased incidence of listeriosis during pregnancy. Infections of human with *L. monocytogenes* has been traced to contaminated foods [4,5]. Once ingested, *L. monocytogenes* is able to cross the intestinal barrier; invasive disease is usually occurred secondary to hematogenous dissemination and typically leads to infection of the placento-fetal unit during pregnancy or to meningitis in immunocompromised patients [6].

This study aimed to evaluate the association of listeria monocytogenes in abortion in a group of Iraqi women.

Methods

A cross-sectional study was designed that included 250 placenta tissues obtained from aborted women attended Al-Imamein Al-Kadhimein Teaching Hospital in Baghdad during the period from June 2014 to November 2015.

Preparation of the samples

Each placenta sample was divided into two parts, each about 50 gm in weight, one stored in 10% formaldehyde solution to use for histopathological study, while the second part cut into small pieces about 15 gm and washed and stored in 5 ml normal slain solution 0.85% to use in bacteriological study.

Preparation of Formalin-Fixed, Paraffin-Embedded tissues (FFPE)

Placental tissue s were sectioned into 3 mm slices and transferred into formalin (10%); fixative volume was 20 times that of tissue on a weight per volume, tissue s were fixed for a minimum 48 hours at room temperature then processed, using gentle agitation, as follows: 70% ethanol for 2 h, 80% ethanol for 2 h, 90% ethanol for 2 hours, absolute ethanol for 2 hours, absolute ethanol for 2 h, xylene for 2 h, xylene for 2 h, first paraffin at 58 °C for 2 h, second paraffin at 58 °C 2 h.

Embedding tissues in paraffin blocks

Small amount of molten paraffin was put in mold, then transfer tissue into mold, placing cut side down, as it was placed in the cassette. Then the cassette was filled with paraffin and left to completely cooled and hardened, from each block, one section of 5µm thickness was taken and stained with Hematoxylin and Eosin for the histopathological diagnosis.

Isolation of *L. monocytogenes*

Placental samples were cuts into small pieces about 15-20 gm and then washed using normal saline solution 0.85%, then put in sterile tubes containing 5 ml normal saline solution 0.85%, tube contents were homogenized by mixing them thoroughly for 10 min to release the bacteria to the solution. Then, 0.5 ml of the placental suspension was inoculated in a sterile tube containing 10 ml of brain heart infusion broth and incubated at 37 °C for 24 h, then 0.5 ml of the bacterial growth was then inoculated on blood agar, nutrient agar, and PALCAM agar and incubated at 37 °C for 24 h.

Laboratory diagnosis

The isolation and identification of *L. monocytogenes* were performed according to Collee et al. 1996 and McFaddin, 2000 [7,8].

Statistical analysis

The statistical analysis of this prospective study performed with the statistical package for social sciences (SPSS) 21.0 and Microsoft Excel 2013. Numerical data were described as mean and standard error. Independent t-test used for comparison between groups. While, categorical data described as count and percentage, Chi-square test used to estimate the association between variables. The lower

level of accepted statistical significant difference is P value below to 0.05.

Results

Out of two hundred and fifty placental samples only fifteen isolates of *L. monocytogenes* were isolated and identified, the total number of the positive *L. monocytogenes* samples constitutes only 6% of the total number of examined placental tissues, the distribution of the isolates according to the age groups of the aborted women revealed that the age group (25-29) years represented the highest number concerning *L. monocytogenes*, which was 12 (80%), while the age group (30-34) years constituted the smallest number concerning this bacteria, which was 3 (20%), (Table 1), however no statistical significant differences between patients' age groups concerning *L. monocytogenes*, in which P value was (0.099). Distribution of samples according to the time of gestation showed that the highest percentage of *L. monocytogenes* was 9 isolates (60%), which were isolated from aborted women in the first trimester while 5 isolates were collected from those in the second trimester (33.3%) and only 1 listeria isolate was isolated from aborted women in the third trimester (6.7%).

Table 1. The distribution of placentitis due to Listeria monocytogenes according to the age groups

Age group (years)	Number of samples	Number of *L. monocytogenes* isolates	Percentage
20-24	33	0	0.0
25-29	127	12	80.0
30-34	75	3	20.0
35-39	15	0	0.0
Total	250	15	6.0
P value		0.099	

Table 2 shows that there is no statistical significant difference between the percentage of isolated *L. monocytogenes* and the time of gestation, in which P value was (0.689), however, the distribution of samples according to the number of abortion was unevenly, in which 12 isolates were isolated from women with a first abortion while only 3 isolates from those with recurrent abortions.

Table 2. The distribution of *L. monocytogenes* isolates according to the time of abortion

Trimester	Number of samples	Number of *L. monocytogenes* isolates	Percentage
First	171	9	60.0
Second	70	5	33.3
Third	9	1	6.7
Total	250	15	6.0
P value		0.689	

Table 3 shows that the highest percentage of listeria was associated with the first abortion was (80%), while those with recurrent abortion showed only (20%) for the isolation of the bacterium. The results have shown no significant association between number of abortion and isolation of *L. monocytogenes*, (P = 0.869). All obtained placenta samples were examined histopathologicaly and according to the reports of the pathologist. Out of 425 placenta samples only 250 were diagnosed to be placentitis and were adopted for this study. (Figure 1) and (Figure 2).

Table 3. The distribution of *L. monocytogenes* isolates according to the number of abortions

Number of abortions	First abortion	Recurrent abortions	Total
Number of samples	204	46	250
Number of *L. monocytogenes* isolates	12	3	15
Percentage of total listeria isolates	80%	20%	6%
P value	0.869		

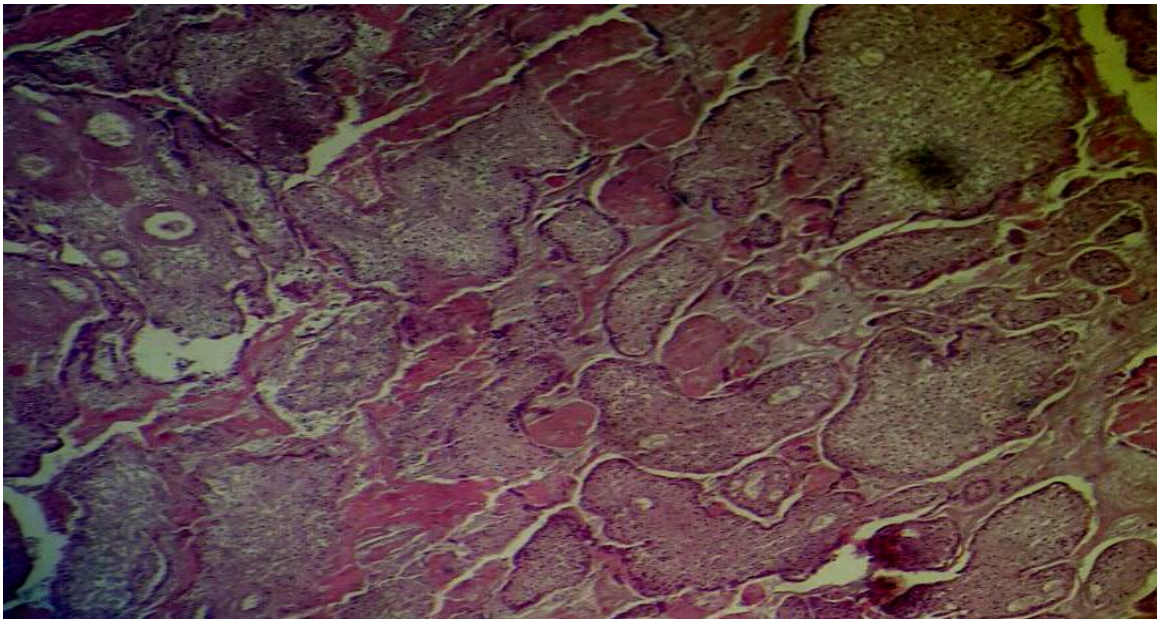

Figure 1. Placenta section showing evidence of placentitis with dense heavy mixed inflammatory cells included both PMN and mature lymphocytes infiltrate intravillous and stromal core (X10).

Discussion

Pregnant women are more susceptible to *L. monocytogenes* infection than healthy individuals; *L. monocytogenes* invades the placenta and result in preterm labor and fetal death [9,10]. During pregnancy, *L. monocytogenes* infection can be asymptomatic or may give rise to subclinical symptoms such as a nonspecific fever, however the development of placento-fetal infection may result in abortion, stillbirth or disseminated neonatal infections, notably granulomatosis infantiseptica [11,12].

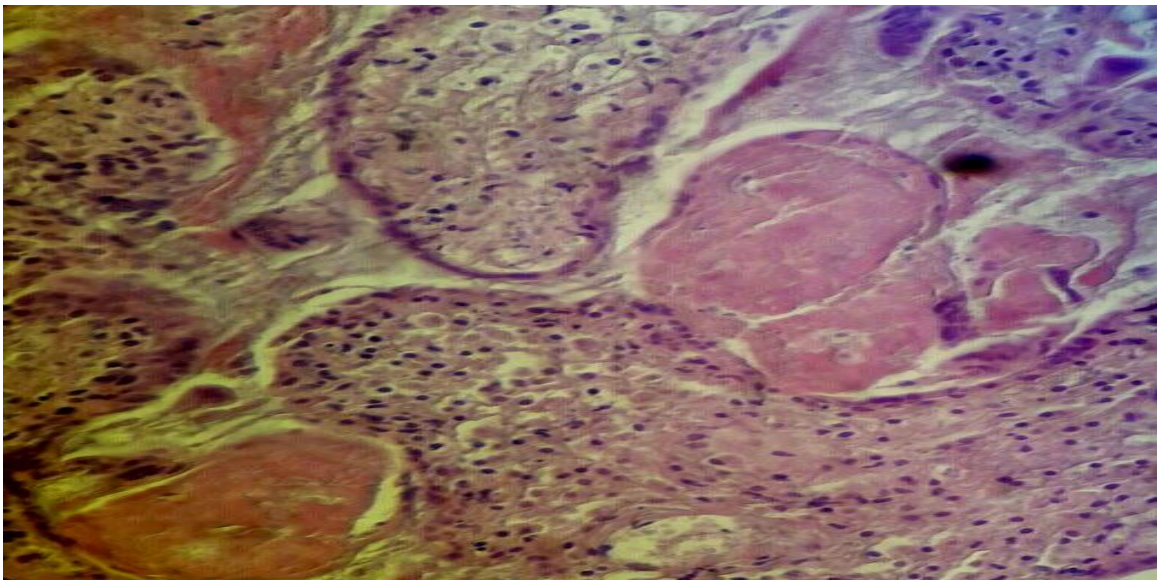

Figure 2. Placenta section showing evidence of placentitis with dense heavy mixed inflammatory cells included both PMN and mature lymphocytes infiltrate intravillous and stromal core (X40).

Jackson et al. in 2010 mentioned that the percentage of *L. monocytogenes* isolated from placenta of pregnant women was 1.6% [(14)]. While Al-Shukri in 2011 found that only five isolates of *L. monocytogenes* out of three hundred three vaginal swaps, where responsible for abortion [(15)]. However, the variation in *L. monocytogenes* percentage might be due to sample size, which was 425 placenta samples, cultivation and identification methods; this study used bacteriological methods while others used molecular methods like nucleic acid assay kits, polymerase chain reaction (PCR) which is rapid but with high cost. Furthermore, the abortion associated bleeding may acts as a cleaning factor that prevents the *L. monocytogenes* colonization [(16)], so we washed the placenta tissue which contains the bacteria to collect them. On the other hand, the cytotoxic T-lymphocytes that found in the placenta tissue were able to recognize and kill the *L. monocytogenes* infected cells by apoptosis, which may lead to the damage of the placental tissue and lead to fetus death [(17)] and this may affect the result because our samples are placenta tissues.

Distribution of the isolates according to the age groups of the aborted women showed that the age group of (25-29) years was the highest among those with listeriosis where the percentage was (80%), while the age group (30-34) years was the lowest where the percentage was (20%), these results agree with Listeria Annual Summary, (2010) which states that the median age of pregnancy-associated cases is 28 years [(18)], but the results disagree with Tahery et al. in 2009 who found the most cases of listeria has been seen in the age group of (41-46) (36%), followed by age groups of (36-40) (27%), (31-35) (13%) and (26-30) [(19)]. These differences in results may due to variation in life style, socioeconomic status; methods of diagnosis, ethnic differences etc…. However, the results of this study have been shown no statistically significances among different age groups and the rate of *L. monocytogenes*, this is agreed with Jamshidi et al. 2009 who mentioned that the seropositivity for *L. monocytogenes* was not age dependent [(20)].

L. monocytogenes was unevenly distributed regarding the time of gestation; however, most isolates were isolated from aborted women at

the first trimester, in which represents the highest percentage of isolation, on the other hand, only one listeria case isolated from aborted women in the third trimester. However, result of the current study disagreed with most of others, most studies showed that the isolation of *L. monocytogenes* during pregnancy is strongly associated with abortion occurred at the third trimester. Listeriosis occurs mainly in the third trimester and this may be due to deficient cell mediated immunity but some cases have been observed at earlier gestational ages, the incidence at lower gestational ages may be underestimated due to reluctance to culture of aborted fetal tissue or products of conception [21], because the doctors don't send samples to the laboratory, however this variation in isolation of the bacteria and time of gestation might be due to the treatment regimen followed by Iraqi gynecologist, which permit antibiotics treatment in wide range during third trimester and since *L. monocytogenes* infection during pregnancy might be asymptomatic or flu-like symptoms so it may miss diagnosed, so listeria infection will be excluded.

Most of listeria cases were associated with the first abortion and only three cases of listeriosis were occurred in those in recurrent abortion. The results also showed that there were no statistical significant differences in association between isolation of Listeria monocytogenes and the number of abortion. Some studies have suggested an association between chronic carriage of *L. monocytogenes* and recurrent abortion, but this suggestion has not been approved [22].

However, negative culture results do not exclude the presence of an infectious organism. *L. monocytogenes* may be difficult to identify in cultures because of bacterial overgrowth and in areas in which multiple organisms may be present [23].

The diagnosis of listeria infection during pregnancy is difficult because most cases are asymptomatic and may lead to abortion and stillbirths furthermore, the continuous failure to isolate the bacterium in blood and tissue cultures, those reasons have been reinforcing the importance of placental histopathological examination to confirm the clinical suspicions [24].

The placenta histological examination showed diffused scattered, tiny, lesions consisting of villous microabscesses with foci of necrosis and peripheral palisaded histiocytes. Focal villitis have been seen with the presence of neutrophils between the trophoblast and the villous stroma.

There were dense heavy mixed inflammatory cells included both polymorphnuclear and mature lymphocytes infiltrate of intravillous stromal core which is a general feature associated with most cases of placentitis, histological findings typical of *L. monocytogenes* infection [25].

In conclusion, this study found that *L. monocytogenes* is associated with some cases of abortion and that the rate of isolation of listeria monocytogenes is not associated with female age, time of gestation or number of abortions.

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Authors Contribution:

Qassim collected the cases, performed the bacterial tests and analyzed the results. Dr. AL Attraqchi helped in the study design and supervising the work. Dr. Khatab participated collection of cases and performed the histopathological examination.

Conflict of interest

The authors declare no conflict of interest.

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