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Original Research Article

Study of listeriosis in spontaneous abortions during preganacy at tertiary care centre

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ABSTRACT

Introduction & Background: Listeriosis is an emerging zoonotic disease. Listeria monocytogenes is an uncommon cause of illness in general population, however, in some high risk groups including neonates, pregnant women, elderly persons, immunosuppressed transplant recipients and others with impaired cell mediated immunity, it is important cause of life threatening bacteremia meningoencephalitis. Listeriosis is 18 times more common in pregnancy (12/100,000) than in the non-pregnant population (0.7/100,000) and 16-27% of all infections with Listeria occur in pregnant women.

Materials and Methods : Isolation of bacteria by conventional methods of microscopy and culture on selective agar PALCUM agar after enrichment of samples by using UVM1, UVM2 .Speciation of listeria done by carbohydrate fermentation test followed by study of pathogenicity by hemolysis on sheep blood agar and CAMP test followed by antimicrobial susceptibility test done.

Result: Out of total 131 patients having history of Spontaneous abortion were screened for Listeria, in that 10(7.6%) were carrying Listeria out of which 3(2.29%) of them found to be positive for L. monocytogenes and 07(5.34%) were other Listeria spp. The prevalence of L. monocytogenes in present study was 03(2.29%) from spontaneous abortion cases.

Conclusion: Based on the results of present study it is concluded that Listeria monocytogenes is responsible for spontaneous abortions in spontaneous abortion during pregnancy in humans.

1. Introduction

Listeria monocytogenes is an uncommon cause of illness in general population However, in some high risk groups including neonates, pregnant women, elderly persons, immunosuppressed transplant recipients and others with impaired cell mediated immunity, it is important cause of life threatening bacteremia and meningoencephalitis.[1] Listeriosis is 18 times more common in pregnancy (12/100,000) than in the non-pregnant population (0.7/100,000)[2] and 16-27% of all infections with Listeria occur in pregnant women.[3]

Pregnant women typically experience only a mild, flu-like illness. However, infections during pregnancy can lead to miscarriage, stillbirth, premature delivery, or life-threatening infection of the newborn.[3] Infection in early pregnancy results in abortion, and the extensive maceration seen at delivery, so soon after the maternal symptoms, suggests that the maternal bacteraemia may result from rather than be the cause of the fetal infection.[4] Fetal infection later in pregnancy causes stillbirth or preterm labour associated with meconium stained liquor and an infected baby.[5]

Present study is carried out to study prospectively, Listeriosis in spontaneous abortion cases during preganancy.

2. Material and Methods

The present research work was carried out in diagnostic laboratory in department of microbiology at tertiary health care hospital

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2.1. Selection of subject

All Pregnant (ANC) mothers attending the obstetric outpatient department with the history of spontaneous abortion were evaluated. Placental bits, endocervical swab were collected with all aseptic methods and processed further as per method described by Donnelly and Baigentwith necessary modifications.[6]

2.2. Enrichment of sample

Under sterile condition placental bits, endocervical swab were inoculated in enrichment media university of vermount (UVM). Inoculated in 45ml of UVM-I then incubated at 37^0C for 18-24h. After incubation, 0.1 ml of UVM-I transfer to the 10 ml of UVM-II and further incubate at 37^0C for 24-36h at 30^0. The enriched inoculum from UVM-II was streaked directly on PALCAM. (Polymyxin, acriflavine, lithium chloride, ceftazidime, aesculin, mannitol (PALCAM). Inoculated petridishes were incubated at 30^oC for 48 hr.

The greenish-yellow glistening, iridescent and pointed colonies of about 0.5 mm diameter surrounded by a diffuse black zone of esculin hydrolysis were considered to be of Listeria. Such colonies were picked and transferred to Brain Heart Infusion Broth and preserved at 4^0C. The isolates in BHI broth exhibiting characteristic tumbling motility at 20-25^{o}C were characterized biochemically and tested for their virulence factors and antibiotic susceptibility. Pathogenic Listerial spp. can be differentiated from non-pathogenic spp. by hemolysis test and CAMP test.

The test was performed by employing disc diffusion method described by Bauer et al[7](1966) using 10 different antibiotic discs procured from Hi Media Lab, India. The diameter of zone of inhibition was measured to nearest millimeter. As the manufacturer has not defined zone diameter break points for Listeria spp. the criteria described by Hansen et al.(2005)[8]was adopted for antibiotic viz. penicillin, ampicillin, gentimicin, erythromycin, vancomycin, tetracycline and chloromphenicol. However, in case of antibiotics like ceftrixone and ofloxacin, the breakpoints defined by manufacturer for Staphylococcus spp. were adopted. The isolates moderately sensitive were considered as sensitive to the antibiotic.

3. Results

Total 131 patients were having history of Spontaneous abortions were evaluated. Out of 131 samples, 120(91.60%) samples belongs to placental bits,11(8.4%) were endocervical swabs.15 -19yr which includes 22(16.43%), 8(21.91%) patients were in the range of 20-24 year, 33 (25.11%) cases were in the age group of 25-29 year, 30(23.28%) patients were in the age group range of 30-34 year, 14(10.50%) patients were in 35-39 age group range and 04(2.73%) patients were above 40 year.

A total of 131 samples of human clinical samples were screened for Listeria, in that 10 were carrying Listeria out of which 3(2.29%) of them found to be positive for L.monocytogenes and 07(5.34%) were other Listeria spp.

All the biochemically confirmed Listeria monocytogenes isolates were streaked on SBA (5%) and haemolysis was observed. A typical ß haemolysis with broad and clear zone was exhibited by isolates.

Isolates confirmed as haemolytic were subjected to CAMP test with staphylococci (MTCC 1144). All the 3 isolates of L.monocytogenes showed characteristic enhancement of haemolytic zone with staphylococcus aureus on SBA.

Isolates were least sensitive to erythromycin (50.00%), chloromphenicol (40.00%), followed by ceftriaxone (10.00%), tetracycline (10.00%). All the isolates were resistant to ceftriaxone (90.00%), tetracycline (90.00%), followed by chloromphenicol (60.00%), erythromycin (50.00%), penicillin (40.00%), less resistant to Ampicillin (40.00%), less resistant to Gentam bjiopycin (40.00%), least resistant to ciprofloxacin (20.00%), vancomycin (20.00%).

In present study, prevalence of L. monocytogenes was found to be 2.29 % in spontaneous abortion cases. The result of this study is in agreement with the earlier reports by Bhujwala et al, (1973),[9] three isolates of L. monocytogenes from 100 spontaneous abortion cases and 3.1% by Dhanshree et al, (2003),[10] from spontaneous abortion cases, highlighting the role of L. monocytogenes as a causative agent of human abortions.

In present study Listeria monocytogenes was isolated from spontaneous abortion cases 03(2.29%) and Listeria spp. 07(5.34%). The present study finding is in agreement with most of the isolates from spontaneous abortion cases in India. Reports of listeriosis from humans are uncertain, either because of failure to identify the isolate, its rarity, improper isolation techniques or lack of awareness.

In present study, 02(18.18%) of Listeria spp. were isolated from endocervical swabs. This finding is in contrast with the Dhanashree et al (2003),[10] in which no isolate were found positive from 69 cervical and 19 vaginal swabs. Also correlated with Manganiello et al,(1991),[11] none of the patient found to harbor the organism in their genital tract, in which endocervical swabs and endometrial tissue were cultured for presence of L. monocytogenes. Present study is similar to the Lida Lotfollahi et al (2011),[12] 3(12%) of Listeria monocytogenes isolated from of bad obstetric history patients.

In present study, L. monocytogenes did not affect the age group ≥40 year. Age range 30-34 years suffered more from spontaneous abortion caused by L. monocytogenes while spontaneous abortion in the age group 25-29 is also affected.

Listeriosis occurs mainly in the third trimester, perhaps due to deficient cell mediated immunity yet, cases have been

Table 1: Incidence of listeria from different samples

S. No.	Name of samples	No. of samples (n=131)	No. of Listeria spp.isolated	No. of Listeria monocytogenes
1	Placental bits	120(91.60%)	0 5(4.16%)	03(2.5%)
2	Endocervical swab	11(8.39%)	0 2(18.18%)	00 (0.0%)
	Total	131	07(5.34%)	3(2.29%)

Table 2: Prevalance of Listeria in different gestational period

Gestational period	Number of patients (n=131)	Listeria spp. (n=7)	L. monocytogenes (n=3)
First trimester	36(27.85%)	04 (11.11%)	02(5.55%)
Second trimester	55(42.00%)	03(5.45%)	01(1.081%)
Third trimester	40(30.13%)	00(00%)	00 (00%)
Total	131	07(5.34%)	03(2.29%)

Table 3: In vitro pathogenicity of Listeria spp

Listeria spp.	Haemolysis on SBA	CAMP test with S.aureus
L.monocytogenes	+	+
L.seeligeri	+	-
L.welshimeri	-	-
L.grayi	-	-

Table 4: Different species distribution in studies

Listeria spp	Placental bits (n=8)	Endocervical swab (n=2)
L.monocytogenes	03 (37.5%)	00(00%)
L.grayi	01(14.28%)	01(50%)
L.innocua	00(00%)	00(00%)
L.welshimeri	02(28.57%)	00(00%)
L.seeligeri	02(28.57%)	01(50%)
Total	08	02

observed at earlier gestational ages. In present study 1st trimester patients were more affected than patients in 2nd trimester.

Present study noted that fever is most commonly presented symptom (70%) then flu like symptoms, upper respiratory tract infections, backache, vomiting headache. Patients can be found to be asymptomatic before spontaneous abortion. This finding matches with Mylonakis et al,(2002)[13] in which 65% of patients were suffered from fever and other symptoms.

Haemolysis is an important characteristic, which is directly related to the pathogenicity of Listeria since non hemolytic Listeria spp. are practically considered as non –pathogenic (Courtieu,1991).[14] The factors involved in the synergistic lysis of RBC and use of CAMP test for identification of L. monocytogenes and L. ivanovii isolates have been reviewed by McKellar (1994).[15] In present study test CAMP test was used to differentiate Listeria monocytogenes from other Listeria spp.

In present study, In vitro antibiogram of L.monocytogenes from human suggest that Vancomycin and Ciprofloxacin should be drug of choice for treating listeriosis. Vancomycin and ciprofloxacin was sensitive to 80% of isolates.

Penicillin, ampicillin, have been used most extensively in the treatment of listeriosis in Penicillin sensitive cases in present studies. High doses are generally used to assure adequate penetration of the umbilical cord and placenta.

4. Conclusions

Present study taking into account the results and intervention findings which concluded that Listeria monocytogenes is responsible for spontaneous abortions in pregnancy in humans. Multidrug resistatance in Listeria monocytogenes is emerging. Vancomycin is drug of choice in successful treatment of listeriosis. It is concluded that for accurate identification of pathogenic species of L. monocytogenes by combination of biochemical characterization and phenotypic characterization and in vitro pathogenicity markers are reliable factors and useful as well. Though routine genital tract culture and treatment are not recommended Nonetheless, although rare and because of the potentially serious consequences, it is important that practicing obstetricians must be familiar with the diagnosis, treatment, and prevention of Listeria infection. Mild, flu-like illness, fever should not be overlooked by Clinician

in pregnant patients, this infections during pregnancy can lead to miscarriage, stillbirth, premature delivery, or life-threatening infection of the newborn.

5. Conflicts of Interest

All contributing authors declare no conflicts of interest.

6. Source of Funding

None.

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