



Original Research Article

Leptospirosis in pregnancy: Prevalence and outcome

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ABSTRACT

Introduction: Leptospirosis is considered as most widespread zoonotic disease in the world. Infection in pregnant women may be grave leading to severe fetal and maternal mortality. The presentation may mimic other viral, bacterial and parasitic infections, acute fatty liver, pregnancy-induced hypertension, and HELLP syndrome.

Objective: Diagnosis of Leptospirosis by ELISA and multiplex PCR for sec Y genes in antenatal patients with fever of 15 days duration and to assess the prevalence of Leptospirosis in the pre-determined group. To compare outcome of pregnancy in the predetermined groups.

Materials and Methods: The study was conducted in the Department of Microbiology and Department of Obstetrics and Gynaecology and necessary required expertise from the Department of Medicine, JNMCH, AMU for a period of 3 years, from September 2016 to August 2018.

Pregnant females attending the Antenatal Clinic during 9 months of pregnancy were included in the study and followed for fetal outcome.

Laboratory diagnosis of leptospirosis was done by IgM ELISA Pan BIO and MULTIPLEX PCR for Sec Y genes.

Result: In our study ELISA test was performed on 130 suspected antenatal female patients for Leptospirosis. It was observed that out of 130 cases, ELISA was reactive in 14 (10.76) patients and non-reactive in 116 (89.23) patients while none of the patient in control was reactive by ELISA. Patients were followed for outcome of pregnancy. Most of the patients delivered by vaginal route 7 (50%), 3 (21.42%) underwent cesarean section, 3 (21.42%) cases had abortion and 1 (7.14%) patient gave stillbirth. Birth weight of live babies born to antenatal females suspected of Leptospirosis categorised into 0 (0%) babies were Extremely Low Birth Weight (ELBW) (<1 kg), 0 (0%) Very Low Birth Weight (VLBW) (<1.5kg), 3 (30%) were Low Birth Weight (LBW) (<2.5 kg), 6 (60%) Normal for Gestational Age (2.5-<4 kg), 1 (10%) Large for Gestational Age (LGA) (≥4 kg).

Conclusion: The hazards of leptospirosis during pregnancy include intrauterine infection with fetal death and abortion, stillbirth, premature labour and signs of congenital leptospirosis within a week or two of delivery and unwanted complications like pregnancy induced hypertension, hypertension, HELLP syndrome leading elective caesarean section.

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1. Introduction

Leptospirosis is supposed to be the most widespread and prevalent zoonotic disease in the world.¹ Globally, it poses

an increasing public health problem, as evidenced by markedly increasing incidence rates and multiple outbreaks in all continents.² Yet, the disease is severely neglected and hence, its global burden is largely unknown. The estimated incidence of about half a million severe human cases annually is probably an underestimation while the

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burden for animal health is unknown.³

Leptospirosis is (re-)emerging globally and numerous outbreaks have occurred worldwide during the past decade. The most recent examples are the epidemics in Bombay 2015, Qaemshahr, Iran each affecting several thousands of people and causing hundreds of deaths.^{4,5} However, the true spread and increase of leptospirosis remains unknown, as the quality and availability of diagnostic tests, testing facilities and surveillance systems are highly variable and frequently absent. Leptospirosis is difficult to diagnose both in the clinic and the laboratory. Therefore, the disease is frequently not recognized and consequently severely neglected.

The main route of infection by these spirochetes is probably by transmission through indirect contact with leptospire secreted into the environment. Most mammalian species are natural carriers of pathogenic leptospire.^{6,7} These include rat, feral, semi-domestic and farm and pet animals as important infection sources.⁸ The risk of acquiring leptospirosis is associated with contact with these animals. Therefore, leptospirosis is an important occupational disease, especially affecting farmers, slaughterhouse workers, pet traders, veterinarians, rodent catchers and sewer workers. Pathogenic leptospire survive longer in a warm and humid environment. Hence, the disease is particularly prevalent in wet tropical and subtropical regions.^{6,7}

In a few cases it may manifest as multiorgan failure where mortality can go upto 40%. Infection in pregnant women may be grave leading to severe fetal and maternal mortality. The presentation may mimic other viral, bacterial and parasitic infections, acute fatty liver, pregnancy-induced hypertension, and HELLP syndrome. Owing to the unusual presentation, leptospirosis in pregnancy is often misdiagnosed and under-reported.⁹

There are several reports from different parts of country on prevalence of *Leptospirosis* in different risk groups. But data is lacking in pregnant females.

This study was planned to assess the prevalence of *Leptospirosis* in the pregnant females and to assess its impact on pregnancy and fetal outcome.

2. Materials and Methods

The prospective study was conducted in the Department of Microbiology and Department of Obstetrics and Gynaecology and necessary required expertise from the Department of Medicine, JNMCH, AMU for a period of 2 years, from September 2016 to November 2017. A detailed clinical and antenatal history was taken from antenatal patients presenting with fever on the basis of predesigned performa based on modified Faine's criterion. The study was approved by the Institute ethical committee, JNMCH, AMU.

2.1. Study groups

After obtaining written informed consent 130 antenatal patients were enrolled in the study into the following three groups:

Group 1: Antenatal females with fever due to Leptospirosis

Group 2: Antenatal females with fever due to causes other than Leptospirosis

Group 3: Antenatal females without fever, without any high risk.

Detailed clinical and socio-demographic history was taken from all patients on the basis of modified Faine's criteria. Patients were followed for the outcome of pregnancy

2.2. Exclusion criteria

1. Co-infection of Leptospirosis with malaria, dengue, typhoid or any other known cause of fever.
2. Patients with bad obstetric history.
3. Patients with other co-morbid conditions.
4. Patients refusing to give consent.

2.3. Serological tests

The leptospire immunoglobulin M (IgM) ELISA (PANBIO IgM ELISA, Standard Diagnostics, Korea): ELISA was done weekly for all the serum samples using commercially available kits according to the manufacturer's instructions. The results were interpreted according to manufacturer's instructions, i.e. values < 9 PANBIO ELISA units were considered negative, 9–11 equivocal, and >11 positive.

2.4. Polymerase chain reaction

2.4.1. DNA isolation

DNA was extracted from serum samples using QIAamp DNA blood mini kit (Qiagen, Germany).

2.4.2. Amplification of DNA

The amplification of DNA was performed in a total volume of 25 μ l.

The primers (Gravekamp C et al.,1993) used for PCR were:

- G1 5'- CTG AAT CGC TGT ATA AAA GT-3' &
G2 5' - GGA AAA CAA ATG GTC GGA AG-3'

The program for amplification included 35 cycles of 94°C (denaturation) for 1 min, 55°C (annealing) for 1 min and 72°C (extension) for 2 min and a final extension step at 72°C for 7 min. The PCR was performed in a final reaction volume of 25 μ l containing 5 μ l of 10x assay buffer [10 mM Tris HCl (pH 9.0), 1.5 mM MgCl₂, 50 mM KCl and 0.01% Gelatin], 200 μ M each dNTPs, 20 pM of each primer, 0.5 U of Taq DNA Polymerase, and template DNA (10 μ l). The PCR products were loaded in 1% wt/vol

agarose gel prepared in TAE (tris base, acetic acid and EDTA- pH 8.0) buffer and detected by ethidium bromide staining after electrophoresis (BioRad, USA). Amplification of 285 base pair DNA fragment was considered as positive for leptospiral DNA.

2.5. Statistical analysis

Statistical analysis was performed with the IBM SPSS Statistics 25. Sensitivity and specificity was calculated by chi square test.

3. Results

A total of 130 patients were included in the study on the basis of clinical history and examination, of which antenatal female patients were divided into socioeconomic groups using Kuppuswamy's Socio-Economic Status Scale revised according to Income Parameter for 2014. According to this index out of 130 suspected antenatal patients, 69 (53.3%) belonged to low socioeconomic status, while 52 (40%) belonged to mid socioeconomic status and 9 (6.92%) to high socioeconomic status.

Out of total 130 suspected cases maximum cases were seen in the month of July and September 20 (15.3%) each, 17 (13%) cases in August and 13 (10%) cases in October. Lowest cases were seen in the month of December 2(1.5%).

Suspected patients diagnosed as having Leptospirosis on the basis of Part A + Part B of Faine's criteria. It was seen that only 12 (9.29%) out of total 130 cases were presumed to be having the disease.

The most consistent symptom seen in this study was fever in 121 (80.6%) cases followed by headache in 92 (93%), vomiting 92 (70.69%), arthralgia 79 (60.6%), lymphadenopathy 35 (26.42%), abdominal pain 31 (23.84%), myalgia with muscle pain 29 (22.3%), cough 42 (32.3%), pitting o edema 15 (11.53%), splenomegaly 13 (10%), maculo-papular rashes 9 (6%), bleeding manifestation 5 (3.84%), and oliguria 9 (6.92%) (Figure 1).

A cute febrile illness was found in 98 (75%) cases, 19 (14.6%) cases were of acute renal failure and 13 (10%) cases had acute icteric illness.

Leukocytosis 64 (56.92%) appeared to be most frequently encountered altered laboratory investigation followed by anemia 59 (45.3%), deranged AST, ALT 19 (14.7%), thrombocytopenia 17 (13.07%), raised serum creatinine 18 (13.8%) and hyperbilirubinemia 13 (10%).

In this study ELISA test was performed on 130 suspected antenatal female patients for Leptospirosis. Table 1 shows result of ELISA. It was observed that out of 130 cases, ELISA was reactive in 14 (10.76%) patients and non-reactive in 116 (89.23%) patients while none of the patient in control was reactive by ELISA.

The results of ELISA were confirmed by PCR. It was found that out of 14 ELISA reactive patients 13

(92.85%) showed positive result while 1 (7.14%) patients was negative by PCR. None of ELISA non-reactive patients was positive by PCR.

It was observed that out of 14 ELISA reactive patients 8 (57.1%) were in 1st trimester at the time of diagnosis, 4 (28.5%) and 2 (14.28%) were in 2nd and 3rd trimester respectively Table 2.

Patients were followed and outcome of pregnancy was noted. Most of the Leptospirosis positive patients delivered by vaginal route 7 (50%), 3 (21.42%) underwent cesarean section, 3 (21.42%) cases had abortion and 1 (7.14%) patient gave stillbirth (Figure 3). Out of 10 live babies born to antenatal females suspected of Leptospirosis 6 (60%) were Normal for Gestational Age(2.5-<4 kg), 3 (30%) were Low Birth Weight(LBW) (<2.5 kg), 1 (10%) Large for Gestational Age (LGA) (≥ 4 kg) (Figure 4).

Rat infestation 12 (85.71%) was found to be the most common epidemiological risk factor found in patients of Leptospirosis, followed by use of public water for bathing in 10 (71.42%), wet surrounding 9 (64.28%), animal contact 7 (50%), walking bare foot in water logged area 4 (28.57%), cleaning activity 2 (14.28%) and 2 (14.28%) patients working in fields were other risk factors observed in the study described in Figure 2.

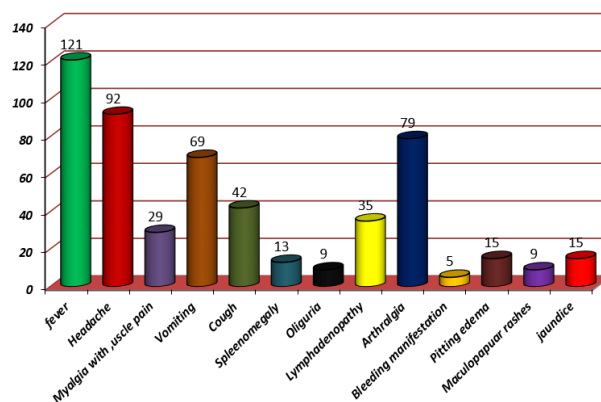


Fig. 1: Clinical symptoms of antenatal patients suspected of *Leptospirosis*

4. Discussion

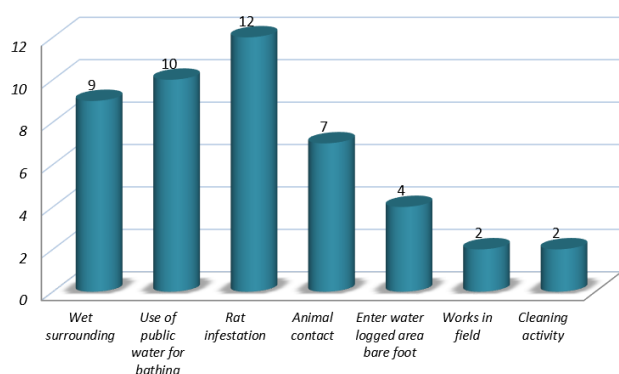
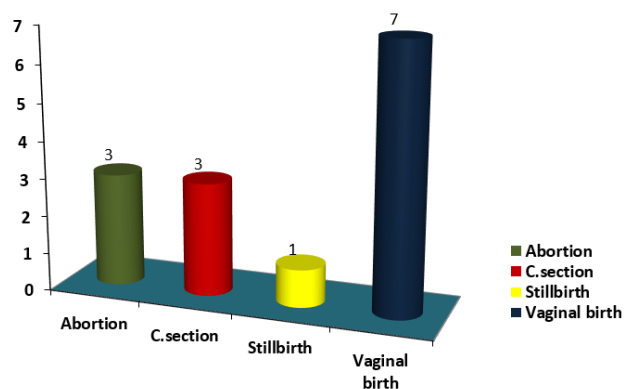
The prospective study was conducted in the Department of Microbiology and Department of Obstetrics and Gynaecology and necessary required expertise from the Department of Medicine, JNMCH, AMU. A detailed clinical history was recorded from each indoor and outdoor antenatal female patient suspected of Leptospirosis in pregnancy, its outcome and Immunological Prognostic Indicators. Detailed clinical and socio-demographic history was taken from all patients on the basis of modified Faine's criteria. Patients were followed for the outcome of

Table 1: Results of ELISA test performed on suspected patients of Leptospirosis (n=130)

	Test	Control	Total	Total
Number of patients tested	130	20	130 (100%)	20(100%)
Reactive	14	0	14 (10.76%)	0 (%)
Non- reactive	116	20	116 (89.23%)	20 (100%)

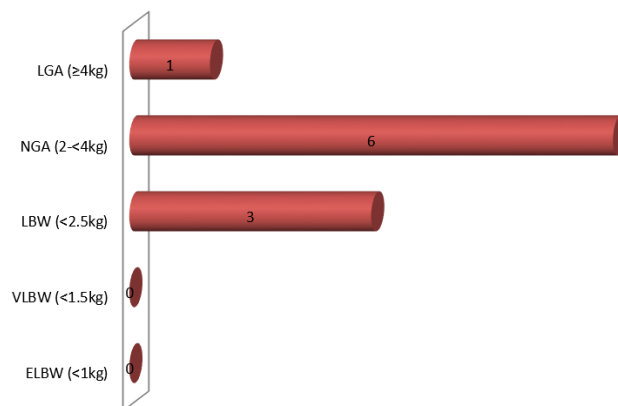
Table 2: Trimester wise distribution of ELISA reactive patients (n=14)

Epidemiological Factors	Rainfall	Contact with contaminated environment	Animal contact
Number of patients	10 (71.43%)	7 (50%)	9 (64.28%)
P value	0.045	0.008	0.025

**Fig. 2:** Epidemiological risk factors found in ELISA reactive patients of *Leptospirosis***Fig. 3:** Outcome of pregnancy in ELISA reactive patients. (n=14)

pregnancy.

Leptospirosis is an acute bacterial infection caused by spirochetes belonging to genus leptospira that can lead to multiple organ involvement and fatal complications.¹⁰ Leptospirosis is considered as the most widespread zoonosis in the world.¹¹ It has a wide geographical distribution and occurs in tropical, subtropical and temperate climatic zones Infection in pregnant women may be grave leading to severe fetal and maternal morbidity and mortality.

**Fig. 4:** Birth weight of live babies born to mothers reactive for *Leptospirosis*

The presentation may mimic other viral, bacterial, and parasitic infections, acute fatty liver, pregnancy-induced hypertension, and HELLP syndrome.

The occurrence of leptospirosis reflects the complex interaction between man, the animal hosts and the environment. Socioeconomic status, occupation, association with animals, recreational activity, climate and rainfall are all linked to the occurrence of leptospirosis.

Fever was found to be most common symptom in the study followed by headache arthralgia, vomiting, cough, lymphadenopathy, abdominal pain, muscle pain, pitting oedema, jaundice, splenomegaly, oliguria and maculopapular rashes.

Leptospirosis has been a significant problem in lowlying areas of India that are densely populated and prone to flooding and water stagnation during monsoon.¹² In our study, majority of cases were seen during the monsoon and post monsoon months and minimum number of cases were reported in December.

Incidence of disease is multifactorial, other factors like exposure to wet fields, bathing in unprotected water bodies, entering water logged area barefooted, indulge in cleaning activities are also important risk factors found in our study. This finding of our study is supported by study d one

by Sehgal that, intimate contact with animals, unprotected entry into waterlogged fields, and bathing in contaminated community ponds are a part of rural life in across north India and a risk factor for Leptospirosis.¹³

Since this is the first study in India for prevalence of Lep tospirosis in antenatal females comparative data is not available. Out of total 130 antenatal females suspected of Leptospirosis, 14 (10.76%) were found positive by ELISA and PCR. However prevalence of 11.7% was reported amongst general population in North India.¹⁴ Authors have reported prevalence ranging from 14-26.9% amongst general population.

In our study 8 (57.1%) cases were from first trimester (p value 0.001) and 4 (28.5%) belonged to second trimester. Only 2(14.28%) cases were reported from third trimester. Higher number of cases were reported from first and second trimester of pregnancy because patients are more exposed to external environment during early pregnancy. During the last trimester since the mobility of patient decreases the exposure to the external risk factors of Leptospirosis also decreases. This may be a reason for lower number of cases during last trimester of pregnancy.

To the best of our knowledge, this is the first study in antenatal patients with Leptospirosis. There are a few case reports on leptospirosis in pregnancy and its fetal outcome. Because it mimic viral, bacterial, parasitic infection it's difficult to diagnose leptospirosis in pregnancy. It is a significant cause of abortion in animals. In our study 3 (21.42%) out of 14 laboratory confirm cases of Leptospirosis showed first trimester abortion. In a retrospective study by Shaked 15 previously reported cases of leptospirosis in pregnancy were reviewed, out of these cases 8 women with Leptospirosis had abortion and it was noted that women are more likely to spontaneously abort if Leptospirosis occurs in the early months of pregnancy.¹⁵

One (7.14%) case in our study had intrauterine fetal death. Aker reported a case of Leptospirosis in a 24-year-old dairy farm worker with a second-trimester intrauterine death.¹⁶ Coghlan reported an attack of leptospirosis due to serotype canicola in a pregnant woman was followed in the convalescent period by death of the foetus.¹⁷ Alston and Broom quoted a report by Hiyeda in Japan on a case of abortion of a 4-month-old foetus by a woman suffering from Weil's disease.¹⁸ Chung also referred to the high rate of abortion and miscarriage that occurs when leptospirosis is acquired during pregnancy in certain rice-growing areas of China.¹⁷

Three (21.42%) out of 14 laboratory diagnosed cases underwent elective caesarean section because of complications like oligohydramnios in first, pregnancy induced hypertension in second, HELLP syndrome in the third patient.

Seven (50%) cases had full term normal delivery. This finding of our study was supported by a review of Chedraui showed that more than 90% of symptomatic mothers have

mild disease and full recovery occur.¹⁹

Out of 10 live birth in patients with Leptospirosis, 6 (60%) babies had Normal weight for Gestational Age (NGA), 3 (30%) were Low Birth Weight (LBW) babies. 1 (10%) baby was Large for Gestational Age (LGA), but this patient had gestational diabetes which may be the primary cause of higher birth weight. None of the babies born to patients with Leptospirosis were Extremely Low Birth Weight (ELBW) and Very Low Birth Weight (VLBW).

5. Conclusion

According to this study, Leptospirosis may be a risk factor for complications during pregnancy and also for poor fetal outcome. More studies with larger sample size are required to assess the exact role of Leptospirosis during pregnancy.

1. The prevalence of Leptospirosis in antenatal females in Aligarh and its peripheral region is 10.76%.
2. Early pregnancy is more prone to infection with Leptospirosis. Also the fetal outcome is unfavourable if patient get infection in early pregnancy.
3. The hazards of Leptospirosis during pregnancy include intrauterine infection with oligohydramnios, PIH, HELLP syndrome, fetal death, abortion, premature labour therefore it must be kept in differential diagnosis in cases of antenatal patients presenting with acute febrile illness and bad obstetric history.
4. The increased awareness among gynaecologist of clinical manifestations Leptospirosis and timely laboratory diagnosis will help reduce morbidity and mortality associated with disease.

6. Limitations

Sample size in the study is less which may be confounding factor. Similar studies with large sample size should be done.

7. Acknowledgments

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8. Source of funding

None.

9. Conflict of interest

None.

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