COMPLETE BLOOD COUNT, IgM AND URINE ROUTINE IN LEPTOSPIROSIS AND THEIR ASSOCIATION WITH SEVERITY IN AN ENDEMIC AREA

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ABSTRACT

BACKGROUND

Leptospirosis is an important cause of mortality and morbidity in India. The present study is conducted to evaluate the association of various blood parameters and urinary parameters with the severity of leptospirosis.

MATERIALS AND METHODS

Our descriptive study enrolled 84 participants with leptospirosis aged 18 years or above admitted under General Medicine in a period of 2 years. Participants with pre-existing renal, hepatic and respiratory diseases were excluded. Study was approved by Institutional Ethics Committee and written informed consent was obtained from all study participants. All investigations were done in the central laboratory of the institution and results were analysed using R®. Parameters are expressed as categorical variables and association was determined using Chi-square test and Fischer's exact test and $p < 0.05$ was considered statistically significant.

RESULTS

Among the study participants, 71% were males and 70% had severe leptospirosis. 72% participants had normal leukocyte count, 70% participants had neutrophilia and 32% participants had lymphocytosis. 66% had thrombocytopenia and 2.4% participants had platelet count less than 10,000 cells/µL. 89% participants had elevated erythrocyte sedimentation rate (ESR). 23.8% participants had more than 5 pus cells in urine and 69% had granular cast in urine. Significant association was observed with severity of leptospirosis for platelet count ($p<0.001$), ESR ($p=0.01$; OR: 5.9, 95% CI: 1.3–25.9), pyuria ($p=0.03$; OR: 5, 95% CI 1.1–23.7) and granular cast in urine ($p<0.001$; OR: 0.6, 95% CI 0.4-0.7).

CONCLUSION

Early diagnosis, monitoring of clinical and laboratory parameters and prompt intervention in terms supportive care and antibiotic therapy will help in reducing morbidity and mortality.

KEYWORDS

Leptospirosis, Platelet Count, Erythrocyte Sedimentation Rate, Urine Pus Cells, Granular Cast in Urine.

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BACKGROUND

Leptospirosis is a widespread zoonotic disease prevalent in the tropical and subtropical geographical areas causative agent being the question mark shaped bacteria[1] belonging to phylum spirochete and of species Leptospira. spp. Leptospirosis is considered the most widespread zoonosis since a large number of species can harbour the pathogen in their renal tubules and transmit them through urine.[1] Rodents are the predominant carriers of the disease currently, due to urbanization, population explosion and climatic changes. The burden of leptospirosis is imparted due to the wide range of symptomatology from flu like syndrome to severe life-threatening organ dysfunction making diagnosis difficult. Lack of a proper diagnostic test makes the diagnosis of the disease even more challenging. Occupational and accidental contact occurs among individuals of low socio-economic strata imposing financial burden on the impoverished. Pulmonary haemorrhage[2–6] and acute kidney injury[7] are important causes of mortality and morbidity among leptospirosis patients. The case fatality rates of pulmonary haemorrhage and Weil's disease with acute renal failure are 10 and 70% respectively.[8] Global burden of leptospirosis is 1.03 million and 58,900 fatalities are reported anuually.[9] Breadwinners of the poor socio-economic strata are affected[9] commonly and them succumbing to morbidity and mortality imposes an even greater burden on the family raising the question of sustenance. Due to increased recognition of the mortality associated with the disease and intensive management the

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case fatality rate has reduced but the morbidity associated with leptospirosis has tripled.[9] Owing to the large population, expanding urban slums and poor hygienic conditions**,** leptospirosis imparts significant death toll among Indians.[9] This is aggravated by the lengthy monsoon season which coincides with the rodent breeding season, waste mismanagement and absence of a systematic national leptospirosis prevention and control programme. Due to the dual monsoon in Kerala this disease has significant health impact in Kerala. Since leptospirosis has overlapping symptomatology with other febrile illness such as dengue fever, misdiagnosis or delay in diagnosis is very common. Early diagnosis and prompt intervention with adequate hydration and use of appropriate antibiotics help in reducing the morbidity and mortality. Studies on severe leptospirosis and the association of blood parameters with the severity of leptospirosis are lacking from Kerala and since severe manifestations are very common from Kerala; this study sheds light into a new aspect of a very common disease. This study is conducted to evaluate the association of the parameters of complete blood count, IgM leptospirosis and urine routine examination findings with leptospirosis and its severity.

MATERIALS AND METHODS

Our present descriptive study enrolled 84 participants over 18 years of age with diagnosis of leptospirosis (positive IgM for Leptospira. spp. using ELISA) and satisfying modified Faine's criteria[10] admitted under department of General Medicine, Sree Gokulam Medical College and Research Foundation, Venjaramoodu between 2014 and 2016. Participants with pre-existing renal impairment, diabetic or hypertensive nephropathy, chronic obstructive pulmonary disease, bronchial asthma and alcoholic liver disease were excluded from the study. The study was approved by Institutional ethics committee and written informed consent was obtained from all study participants. Complete blood count [total leukocyte count, differential count, platelet count, erythrocyte sedimentation rate (ESR) and IgM antibodies to leptospira] and urine routine examination findings were collected in separate case record forms. Participants were categorized based on the recorded findings and severity of leptospirosis [mild leptospirosis (acute febrile illness with no complications), severe leptospirosis (acute febrile illness with any one or more of the following such as jaundice, acute kidney injury, pulmonary haemorrhage, ARDS, neuroleptospirosis, thrombocytopenia, myocarditis, ocular complications or hypokalaemic paralysis[10])]. All investigations were done in the central laboratory of the institution using appropriately standardized technique; IgM lepto was detected and quantified using ELISA. Sample size was calculated as 84 using the formula $Z\alpha^2P(100-P)/L^2$, Zα-1.96, P-82, L-10%.[11] Data was analysed using free to use software R® and all parameters are expressed as categorical variables and association was determined using Chi-square test, Fischer's exact test and Odds ratio (OR) was used to express the association in 2x2 tables. $p < 0.05$ was considered statistically significant.

RESULTS

84 participants were enrolled in the study of which 71.4% (n=60) were males and 28.6% (n=24) were females. Among the study participants, 70.2% (n=59) were diagnosed as having severe leptospirosis and 29.8% (n=25) had mild leptospirosis. IgM Leptospira titer were 12-24 Panbio units in 64.3% (n=54), 25-49 in 31% (n= 26), 50-75 in 3.6% (n= 3.6%) and was >75 in 1.2% (n=1) study participants. Among the study participants leukopenia was seen in 1.2% (n=1), normal leukocyte count was seen in 72.6% (n=61), leukocytosis was seen in 26.2% (n=22). The proportion of participants with elevated total leukocyte count is demonstrated in table 1. Neutrophilic predominance was seen in 70.2% (n=59) and lymphocytic predominance was seen in 32.1% (n=27) participants. Distribution of neutrophils and lymphocytes among the study participants are demonstrated in table 2 and table 3 respectively. Thrombocytopenia was seen in 66.6% (n=56) participants and severe thrombocytopenia (platelet count less than 10, 000 cells/μL) was seen in 2.4% (n=2) participants. Distribution of platelet count is demonstrated in table 4. 10.7% (n=9) participants had normal ESR (less than 20mm in the first hour) and 89.3% (n=75) participants had elevated ESR. ESR among study participants is demonstrated in table 5.

Total Leukocyte Count (cells/μL)	n (%)
11001-13000	9 (40.9)
13001-15000	5 (22.7)
15001-17000	4 (18.1)
17001-19000	4 (18.1)

Table 1. Stratified Frequency Table of Participants with Leukocytosis

Neutrophils (cells/100 Leukocytes)	n (%)
40-70	25 (29.8)
71-80	21 (25)
81-90	34 (40.5)
>90	4 (4.8)

Table 2. Stratified Frequency Table of Distribution of Neutrophils among Participants

Lymphocytes (cells/100 Leukocytes)	n (%)
<20	57 (67.9)
20-50	26 (31)
51-60	1 (1.2)

Table 3. Stratified Frequency Table of Distribution of Lymphocytes among Participants

Platelet Count (cells/µL)	n (%)
> 1.5 lakhs	28 (33.3)
1–1.5 lakh	16 (19)
75,000-99,999	11 (13.1)
50,000-74,999	13 (15.5)
25,000-49,999	10 (11.9)
10,000-24,999	4 (4.8)
<10,000	2 (2.4)

Table 4. Stratified Frequency Table of Platelet Count Among Study Participants

ESR (mm in 1st hour)	n (%)
0-20	9 (10.7)
21-40	22 (26.2)
41-60	13 (15.5)
61-80	9 (10.7)
81-100	20 (23.8)
>100	11 (13.1)

Table 5. Stratified Frequency Table Demonstrating Distribution of ESR Among Study Participants

Pus cells in urine were seen in 98.8% (n=83) participants. 1-4 pus cells in urine/high power field (HPF) in urine was seen in 75% (n=63) participants, 5-10 cells/HPF in 20.2% (n=17) participants and more than 10 cells/HPF in 3.6% (n=3) of participants. Comparison of parameters between mild and severe leptospirosis is demonstrated in table 6. 69% (n=58) participants had granular cast in urine. There was no association between plasma IgM levels and severity of leptospirosis (p=0.3; OR: 1.6, 95% CI 0.6-4.5), total leukocyte count and severity of leptospirosis (p=0.4; OR: 1.6, 95% CI 0.5-5), neutrophil count and severity of leptospirosis (p=0.6; OR: 2.5, 95% CI 0.9-6.8), lymphocyte count and severity of leptospirosis (p=0.9; OR: 1, 95% CI 0.4-2.7). There was significant association between platelet count and severity of leptospirosis (p<0.001), ESR and severity of leptospirosis (p=0.006), urine pus cells and severity of leptospirosis (p=0.027) and granular cast in urine and severity of leptospirosis (p=0.001) which are demonstrated in table 7-13 respectively.

Parameter	Mild Leptospirosis n (%)	Severe Leptospirosis n (%)
Total count >11000 cells/µL	5 (20)	17 (28.8)
Neutrophil >70 cells/100 leukocytes	14 (56)	45 (76.3)
Lymphocyte > 50 cells/100 leukocytes	8 (32)	19 (32.2)
ESR > 20 mm in 1st hour	19 (76)	56 (94.4)
IgM Lepto >50 Panbio units	7 (28)	23 (39)
Platelet count <1.5 lakh cells/µL	7 (28)	49 (83.1)
Platelet count <1 lakh cells/µL	0 (0)	40 (67.8)
Pus cells >5/HPF	2 (8)	18 (30.5)
Granular cast	0 (0)	26 (44.1)

Table 6. Frequency Table of Baseline Parameters between Mild and Severe Leptospirosis

Platelet Count (cells/ µL)	Leptospirosis		Total
	Mild	Severe	
< 1.5 lakhs	18	10	28
≥ 1.5 lakhs	7	49	56
Total	**25**	**59**	**84**

Table 7. Association between Platelet Count and Severity of Leptospirosis

There was significant association between platelet count and severity of leptospirosis (p<0.001; OR: 12.6, 95% CI 4.2-38) indicating 12.6 Odds of encountering mild leptospirosis among participants with platelet count <1.5 lakhs.

Platelet Count (cells/ µL)	Leptospirosis		Total
	Mild	Severe	
< 1 lakh	25	19	44
≥1 lakh	0	40	40
Total	**25**	**59**	**84**

Table 8. Association between Platelet Count (Categorized as ≥ & <1 Lakh Cells/ µL) and Severity of Leptospirosis

There was significant association between platelet count and severity of leptospirosis (p<0.001; OR: 0.4, 95% CI 0.3-0.6) indicating 0.4 Odds of encountering mild leptospirosis among participants with platelet count < 1 lakh.

Platelet Count (cells/ µL)	Leptospirosis		Total
	Mild	Severe	
>1.5 lakhs	18	10	28
1-1.5 lakhs	7	9	16
75000-99999	0	11	11
50000-74999	0	13	13
25000-49999	0	10	10
10000-24999	0	4	4
<10000	0	2	2
Total	**25**	**59**	**84**

Table 9. Association of Platelet Count and Severity of Leptospirosis

There was significant association between platelet count and severity of leptospirosis (p<0.001) probably indicating the higher proportion of participants with lower platelet count having severe leptospirosis.

ESR (mm in 1st hour)	Leptospirosis	
	Mild	Severe
< 20	6	3
≥ 20	19	56

Table 10. Association between ESR and Severity of Leptospirosis

There was significant association between ESR and severity of leptospirosis p=0.01; OR: 5.9, 95% CI: 1.3–25.9) indicating 5.9 Odds of encountering mild leptospirosis among participants with ESR <20mm.

ESR (mm in 1st hour)	Leptospirosis		Total
	Mild	Severe	
0-20	6	3	9
21-40	9	13	22
41-60	4	9	13
61-80	4	5	9
81-100	1	19	20
>100	1	10	11
Total	**25**	**59**	**84**

Table 11. Association between Stratified ESR and Severity of Leptospirosis

There was significant association between ESR and severity of leptospirosis (p=0.006) probably indicating the higher proportion of participants with higher ESR having severe leptospirosis.

Pus cells (/HPF)	Leptospirosis	
	Mild	Severe
< 6	23	41
≥ 6	2	18

Table 12. Association between Urine Pus Cells and Severity of Leptospirosis

There was significant association between urinary pus cells and severity of leptospirosis (p=0.03; OR: 5, 95% CI 1.1–23.7) indicating 5 Odds of encountering mild leptospirosis among participants with urinary pus cells <6.

Granular cast	Leptospirosis		Total
	Mild	Severe	
Absent	25	33	58
Present	0	26	26
Total	**25**	**59**	**84**

Table 13. Association between Granular Cast in Urine and Severity of Leptospirosis

There was significant association between granular cast and severity of leptospirosis (p<0.001; OR: 0.6, 95% CI 0.4-0.7) indicating 0.6 Odds of encountering mild leptospirosis in participants without granular cast.

DISCUSSION

71.4% of the study participants were males and 28.6 % were females. Higher incidence of leptospirosis has been previously reported in males[12–15] and can be explained via the higher risk of occupational and recreational exposure among men contacting them with Leptospira infected animals or contaminated water.[16] This finding has been confirmed during the leptospirosis epidemic in Philippines of 1998-2001 where 87% of the participants were males and 70% participants were involved in outdoor activities and 80% had positive history of exposure to sewage or surface water.[16] 72.6% of study participants had normal leukocyte count which is concurrent to previous reports of 53-75% participants with normal leukocyte count.[17,18] Leptospira cause minimal changes in leukocyte counts in the initial week of fever that too only in a small proportion of patients. This could also be due to the peculiar change produced in leukocyte count by Leptospira. Spp., which cause reduction of leukocyte over the initial five days of fever followed by rise over next week.[18] Complete blood count or total leukocyte count is usually done for evaluation of fever between 3rd to 5th days of fever. During this period the typical picture in leptospirosis is normal or lower leukocyte counts, hence this finding. 26.2% participants had leukocytosis which corresponds to proportion of patients with leukocytosis in leptospirosis.[18] Being a tertiary care hospital, unresolved fever of more than 5 days duration are referred to, which coincides with period of elevated leukocyte count in leptospirosis which also explain the proportion of participants with leukocytosis. 1.2% participants had leukopenia which is considerably low compared to previous reports of 16% leukopenia among leptospirosis patients. Leukopenia is thought to be due to the bone marrow suppression[19] by Leptospira. spp., and the difference in observed prevalence is probably due to the variations in serovars in various geographical locations. Though the proportion of participants with neutrophilic predominance and lymphocytosis has not been clearly described, 70.3% participants had neutrophilic predominance (>70 cells/100 leukocytes) and 1.2% of participants had lymphocytosis (> 40 cells/100 leukocytes) in peripheral smear. Neutrophilic predominance is a common blood picture in virulent and severe leptospiral infection[20] and has been reported to steadily increase during the initial two weeks of fever.[18] Lymphopenia is a typical finding acute febrile phase of leptospirosis[18] and is seen in 50% of leptospirosis patients during 5th day of febrile illness and return to normal over two weeks.[18] Thrombocytopenia was seen in 66.6% of the participants, which corresponds to reports of 50-93% patients with leptospirosis developing thrombocytopenia[21] and is due to peripheral platelet consumption as a result of haemorrhage, antibodies destroying platelets and bone marrow suppression.[20,22,23] Elevated ESR was seen in 89.3% participants which has been described as a common finding in leptospirosis.[24,25] This could be due to leukocytosis, reduction of red cell count owing to myelosuppression and coagulation or due to increase in fibrinogen in leptospirosis[26] which inhibits rouleaux formation and cause subsequent increase in ESR. 98.8% participants had pyuria, greater than 5 pus cells/HPF were seen in 23.8% of participants. Lower than 5 pus cells/HPF is considered normal as it can occur due to contamination of sample, incorrect technique of sample collection and also due incorrect timing of the sample collection. Pyuria has been considered a normal finding in leptospirosis since Leptospira colonizes in renal tubules causing activation of macrophages and dendritic cells of the kidneys[27] leading to subsequent activation and recruitment of neutrophils and T-cells which are interpreted as pus cells in urine. Granular casts were seen in 69% of the study

participants which are formed as a result of degeneration of cellular cast or due to aggregation of plasma proteins or immunoglobulins, all of which are possible in leptospirosis. 70% participants were having severe leptospirosis in contrast to reports of 5-15%[1, 28] incidence. This could be due to the higher incidence of leptospirosis in Kerala or due to the wide range of presentation from mild fever to severe organ involvement of leptospirosis, predominant participants attending hospitals would be those with severe leptospirosis. Our study did not demonstrate any association between plasma IgM and severity of leptospirosis, which could be due to lower than required accuracy and sensitivity of the test in detecting immunoglobulin directed against Leptospira.[29–32] No association was observed between leukocyte count and severity of leptospirosis which could be due to varying leukocyte count in leptospira infected individuals. Majority of leptospirosis infected individuals show normal leukocyte count in the initial 5 days of fever followed by an increase over the subsequent days till 2 weeks of fever and ~38% have been reported to develop leukopenia in the initial 5 days of fever. This expunges the credibility of leukocyte count in determining severity of leptospirosis. We did not find any association between differential leukocyte count (neutrophil and lymphocyte count) and severity of leptospirosis. Differential neutrophil count is in the range of low to normal in the initial week of fever which subsequently increase over the second week.[18] Differential lymphocyte count also show a similar pattern though higher proportion of participants show lymphopaenia.[18] Since our study enrolled participants with history of fever ranging from a day to two weeks duration we did not find any association between differential leukocyte count and severity of leptospirosis.

We found significant association between platelet count and severity of leptospirosis. Platelet count reduces over 3-5 days of fever in 56-73% participants[18] and is a result of peripheral consumption of platelets and bone marrow suppression in severe leptospirosis. We found significant association between haematocrit and severity of leptospirosis with higher Odds of encountering mild leptospirosis in participants with low haematocrit. This is in contrast to previous reports of reduced haematocrit[33] among participants with severe leptospirosis.[18] This could be due to the enrolment of higher number of male participants who have higher haematocrit[34] physiologically which could contribute to this association. We also found significant association between urine pus cells, urinary granular cast and severity of leptospirosis with higher Odds of encountering mild leptospirosis in participants with lower urinary pus cell count and lower Odds of encountering mild leptospirosis in participants without granular cast in urine. Renal tubules harbour Leptospira in infected individuals resulting activation of renal macrophages and dendritic cells leading to activation and recruitment of T-cells and neutrophils which are interpreted as urinary pus cells and hence the association. Granular cast are formed as a result of degradation of cellular casts and also due to the immunoglobulins filtered via kidney. This is contrary to the finding expected in leptospirosis and this association could not be explained. Further studies on the association of granular casts in urine and severity of leptospirosis are required to shed light into this finding.

CONCLUSION

Severe leptospirosis was seen in 70.2% participants. 26.2% showed leukocytosis and 1.2% showed leukopenia. 70% had neutrophilia and 32% had lymphocytosis. 67% had low platelet count with 2.4% developing severe thrombocytopenia. Most the participants had elevated ESR and urinary pus cells. Significant association was observed between severity of leptospirosis and platelet count, ESR, urinary pus cells and granular cast. Since leptospirosis is an important cause of mortality and morbidity, early diagnosis, monitoring of clinical and laboratory parameters and prompt intervention in terms supportive care and antibiotic therapy will help in reducing morbidity and mortality.

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