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ORIGINAL RESEARCH

Serological Evidence of Co-infection of Dengue, Leptospirosis and Scrub Typhus in Patients Presenting with Acute Febrile Illness in a Tertiary care Hospital

Rupinder Bakshi[1], Satinder Kaur[2], Tanveer Kaur[2], Ritika Ghai[2], Karashdeep Kaur[2], Ramanpreet Kaur[2], Jaspreet Kaur[2], Palika Sharma[2], Amritpal Kaur[2], Harbhajan Singh[3]

[1]Associate Professor and Principal Investigator, Viral Research and Diagnostic Laboratory, Government Medical College, Patiala, Punjab, India.

[2]Research Scientist, Viral Research and Diagnostic Laboratory, Government Medical College, Patiala, Punjab, India

[3]Technician, Viral Research and Diagnostic Laboratory, Government Medical College, Patiala, Punjab, India.

ABSTRACT

Background: Acute febrile illness (AFI)is specified as sudden onset of fever of unknown origin lasting for 1-14 days. AFI may be caused by pathogens like bacteria, viruses, parasites, and fungi leading to infectious diseases. Dengue is a vector-borne viral disease that is transmitted by Aedes aegypti mosquitoes. Dengue NS1 Ag is released into the blood during viral replication in infected patients and is detectable from the first day after fever. As NS1 Ag can be identified promptly, it acts as a beneficial biomarker for the early detection of dengue, allowing rapid management of dengue fever.

Materials and Methods: A retrospective study was carried out on blood samples of patients presenting with acute febrile illness in OPD/IPD of a tertiary care hospital from September 2019- December 2019. Out of 694 samples, the samples which were 2 mL in quantity, non-hemolytic, non-lipemic, maintained in cold chain along with complete demographic data were selected for the present study i.e. n=270.

Results: Blood samples of 270 patients who presented with acute febrile illness and met with our inclusion criteria were tested for dengue NS1 antigen/IgM antibodies based on their fever history. Out of 270, 120 patients tested positive for dengue.

Conclusion: To conclude, patients in tropical countries presenting in the post-monsoon season with acute febrile illness not responding to appropriate and adequate therapy aimed for a suspected tropical infection such as dengue should be evaluated for concurrent infections with other microorganisms.

Keywords: Dengue, Leptospirosis, Scrub Typhus, Co-Infection, ELISA, Rainfall

Corresponding Author: Dr. Gur paramjeet singh gill, Associate Professor ENT, NC Medical college, Panipat, Punjab, India. Email: gpsgill67@gmail.com

INTRODUCTION

Acute febrile illness (AFI)is specified as sudden onset of fever of unknown origin lasting for 1-14 days. AFI may be caused by pathogens like bacteria, viruses, parasites, and fungi

leading to infectious diseases.[1] Dengue is a vector-borne viral disease that is transmitted by Aedes aegyptimosquitoes.[2] Dengue NS1 Ag is released into the blood during viral replication in infected patients and is detectable from the first day after fever.[3,4] As NS1 Ag can be identified promptly, it acts as a beneficial biomarker for the early detection of dengue, allowing rapid management of dengue fever5. Anti-dengue IgM antibodies appear about 4 to 6 days after the onset of fever and remain in circulation for about 2 -3 months. So in the latter case, dengue infection can be identified using dengue IgM ELISA.[4] Bacterial zoonotic infections, leptospirosis, and scrub typhus are not very common in Punjab. The incidence of these infections is high in tropical and subtropical areas.[5] Due to this reason, missed diagnosis of both of these infections may lead to absenteeism of antibiotic therapy affecting morbidity. Scrub typhus is because of Orientia tsutsugamushi that belongs to the family Rickettsiaceae. A bite from the trombiculid mite spreads scrub typhus, Leptotrombidium delicenses, which is a parasite of rodents and is found in heavy scrub vegetation areas. It transmits the bacteria from infected rodents to other rodents and humans,[6] O. tsutsugamushi, when introduced in the body, proliferates and damages the small blood vessels leading to leakage of fluid, platelet aggregation, inflammation, and micro-infarction.[7] An ulcer is formed at the site of the bite, followed by other symptoms like fever, a spotted rash, and swelling of lymph glands. Generally, the illness lasts for 10 to 12 days; if left untreated, death may also occur.[8,9] Leptospira interrogans cause leptospirosis. It is spreads through exposure to the urine of infected animals, especially rodents, either directly or from contact with soil or water contaminated by the urine. This bacterium remains for extended periods in the kidney tubes in infected animals. It is excreted in the urine of such asymptomatic animals for many years, causing animal to animal or animal to human transmission.[10] The usual portal of entry is through abrasions in the skin or via the conjunctiva. Infection may also take place via intact skin after prolonged immersion in contaminated water. Water-borne transmission of leptospirosis has also been documented.[11] During the rainy season, the incidence of this infection increases. The onset of this infection is usually sudden, with symptoms including fever, muscle pain, headache, conjunctival infections, abdominal pain, and meningitis. Sometimes serious complications such as hepato-renal failure and central nervous system involvement may arise due to delayed diagnosis and treatment.[12] The incubation time of this disease is usually one to two weeks. After 3 days of initial infection, IgM antibodies appear, and they may persist forup to 5 months.[13] The Indian climate provides conducive conditions for the proliferation of dengue, leptospirosis, and scrub typhus. All these diseases peak during the monsoon and share similar clinical characteristics. This is the reason the presence of coinfection of these diseases is often missed. The clues pointing toward co-infection should be followed diligently. Co-infections may influence disease severity, treatment outcomes, and the development of drug resistance. To differentiate between these viral diseases, the early diagnosis of the patient is a must, so that proper antibiotics must be prescribed for controlling viral transmission.[14]

MATERIALS & METHODS

A retrospective study was carried out on blood samples of patients presenting with acute febrile illness in OPD/IPD of a tertiary care hospital from September 2019- December 2019. Out of 694 samples, the samples which were 2 mL in quantity, non-hemolytic, non-lipemic, maintained in cold chain along with complete demographic data were selected for the present study i.e. n=270.This hospital caters to the needs of patients from all over Punjab and Haryana. These patients were having a fever history of 1-15 days. Serum was separated from blood samples by centrifugation and stored at-20ºC for further analysis. The diagnostic

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procedures performed for these acute febrile illness presenting cases are elucidatedin [Figure 1].

270 samples from the patients with acute febrile illness were processed for dengue testing according to their fever history. The patients having a fever history of 1-5 days were tested for dengue NS1 Antigen using commercially available DengueNS1 Antigen ELISA(RecombiLISA) (CTK BIOTECH, INC. California). The patients having fever history >5 days were tested for IgM antibodies using NIV DENGUE IgM CAPTURE ELISA kit (ICMR- NIV, PUNE). These samples were additionally tested for leptospirosis using Panbio Leptospira IgM ELISA kit (Standard Diagnostics, INC. According to the manufacturer's instructions, the Republic of Korea) and Scrub typhus use InBiOS Scrub Typhus Detect IgM ELISA (Seattle, WA USA).

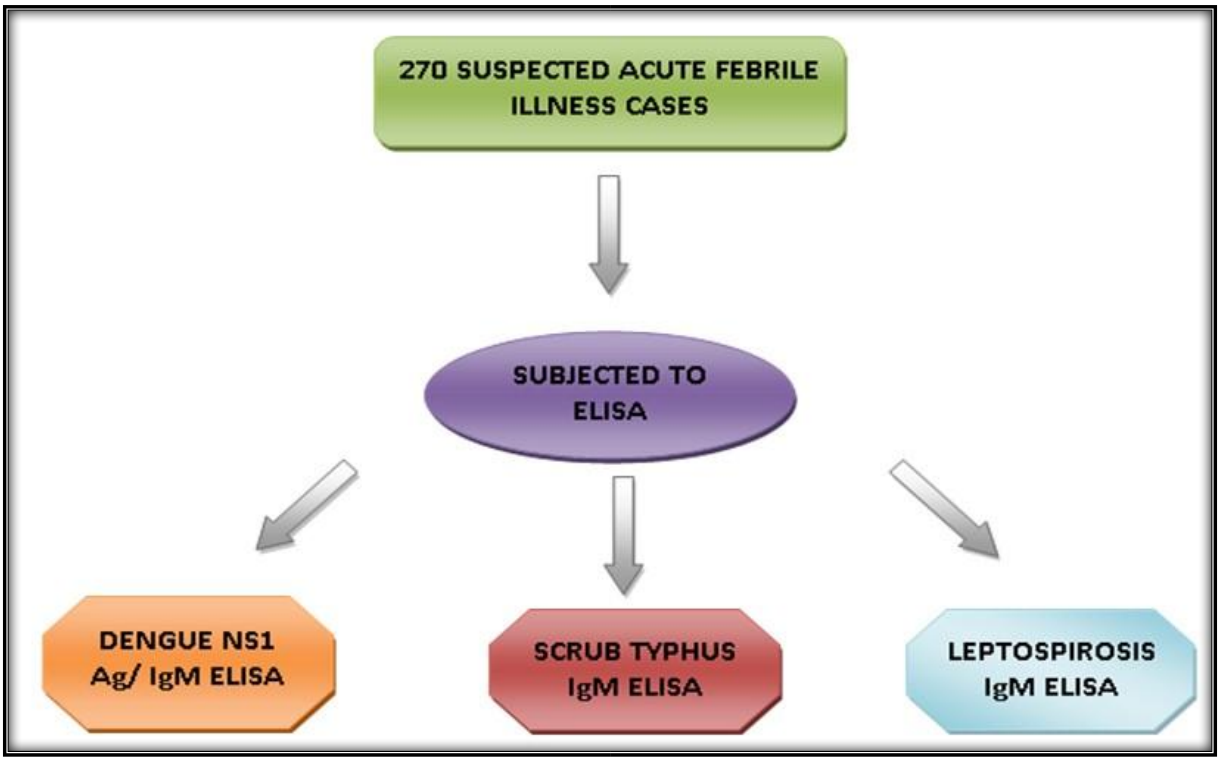

Figure 1: The concise scheme followed for case processing and laboratory analysis

Statistical analysis

Statistical analysis was performed using Graph pad prism software version 5. Categorical variables like gender were compared using Chi-square independent test. Significant variation between other variables was analysed using one-way ANOVA followed by Tukey's test and two-way ANOVA followed by Dunn’s multiple comparison test.

RESULTS

Blood samples of 270 patients who presented with acute febrile illness and met with our inclusion criteria were tested for dengue NS1 antigen/IgM antibodies based on their fever history. Out of 270, 120 patients tested positive for dengue. These 270 samples were further tested for leptospirosis and scrub typhus infections as an underlying cause of acute febrile illness. A total of 10 cases of leptospirosis and 9 cases of scrub typhus were found positive out of 270 patients.Co-infection of dengue and leptospirosis was found in 8 cases while

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coinfection of dengue and scrub typhus was found in 3 cases. No case of mixed infection was reported.

Our study involved 136 male subjects, out of which 74 males were found to be dengue positive, 5 leptospirosis, and 5 males were found to be scrub typhus positive. 52 subjects carried no infections. Out of 134 females, Dengue fever was discovered in 46 females. 5 were positive for leptospirosis and 4 for scrub typhus, and 79 females carried no infection [Figure 2]. The rate of infection for dengue was comparatively higher in males as compared to females.

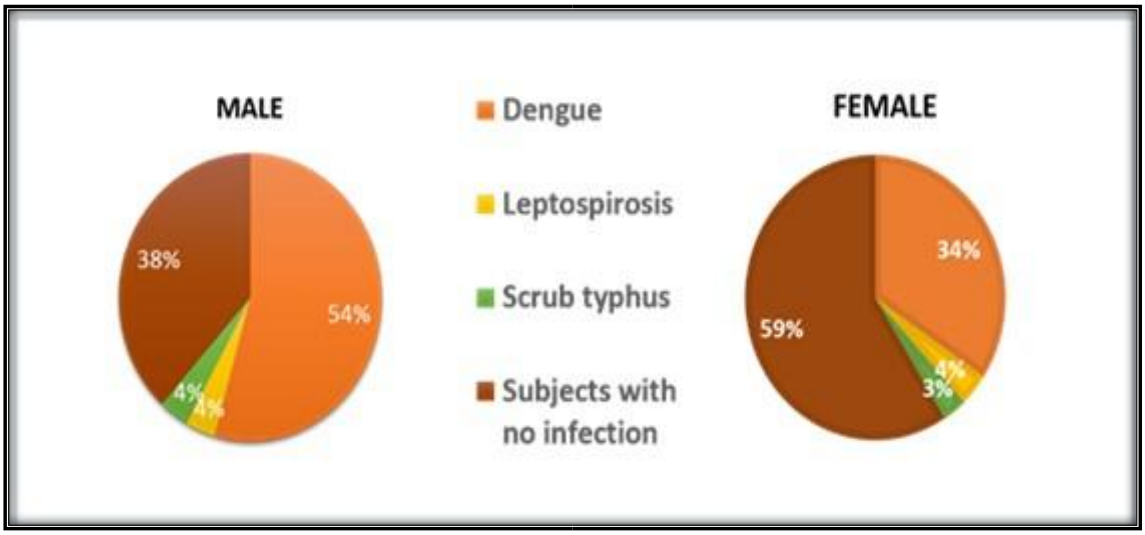

Figure 2: Gender wise distribution of subjects with infection and no infection

The highest prevalence of dengue, leptospirosis, and scrub typhus was found in age groups of 21-40years, followed by 41-60 years. The lowest number of patients were found in the age group of 61-80 years [Figure 3].

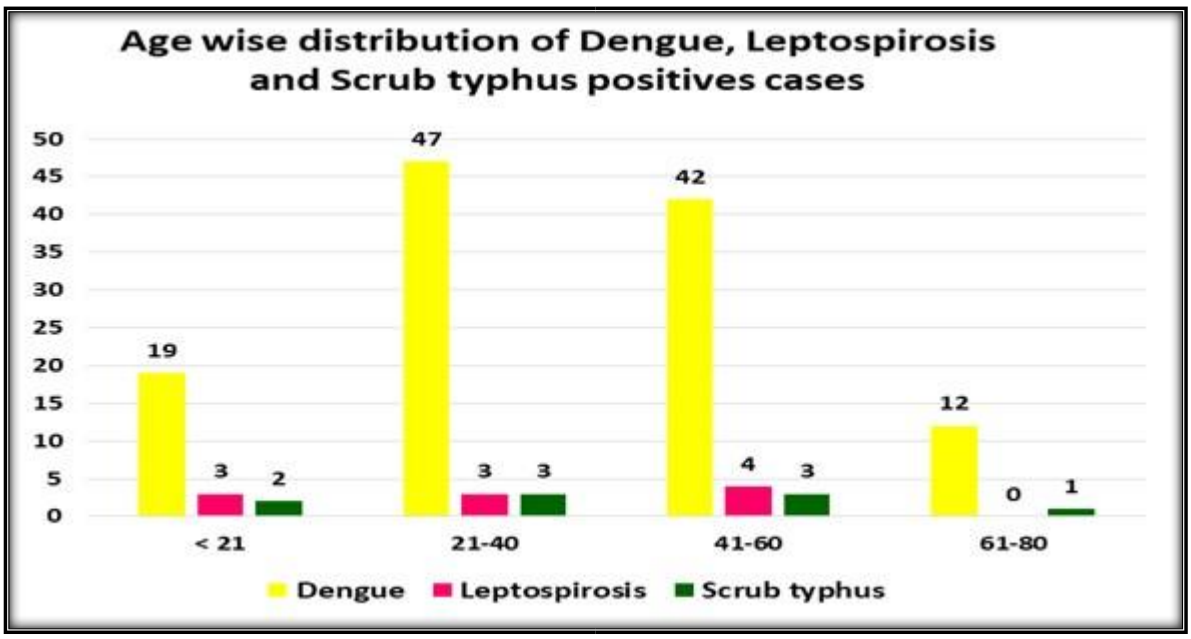

Figure 3: Age-wise distribution of dengue, leptospirosis, and scrub typhus patients

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Information regarding rainfall in the year 2019 in Punjab was fetched from rainfall Statistics of India- 2019 by the India Meteorological Department (Ministry of Earth Sciences) through report no.MoES/IMB/HS/Rainfall Report/ 01(2021)/57. The highest rainfall was recorded in July- October.

Most studies have reported dengue and leptospirosis cases in the post-monsoon season. Our study recorded the highest dengue, leptospirosis, and scrub typhus cases in October and September, followed by November and December. There were no leptospirosis cases in the month of November and no scrub typhus cases in the month of December (Fig.5). This can be correlated to rainfall data analyzed [Figure 4].

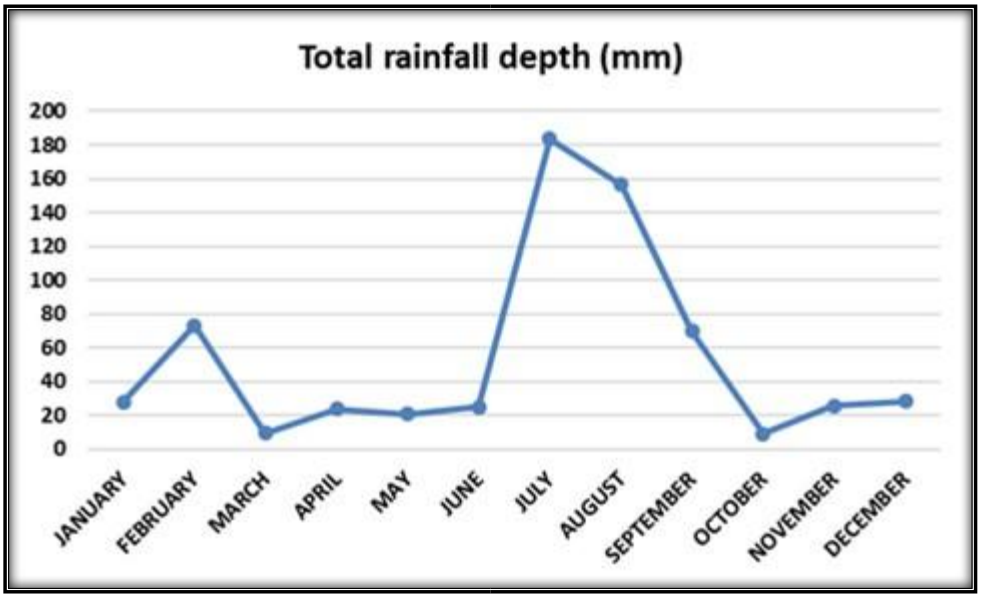

Figure 4: Total rainfall depth recorded in one year in different districts covered under the study

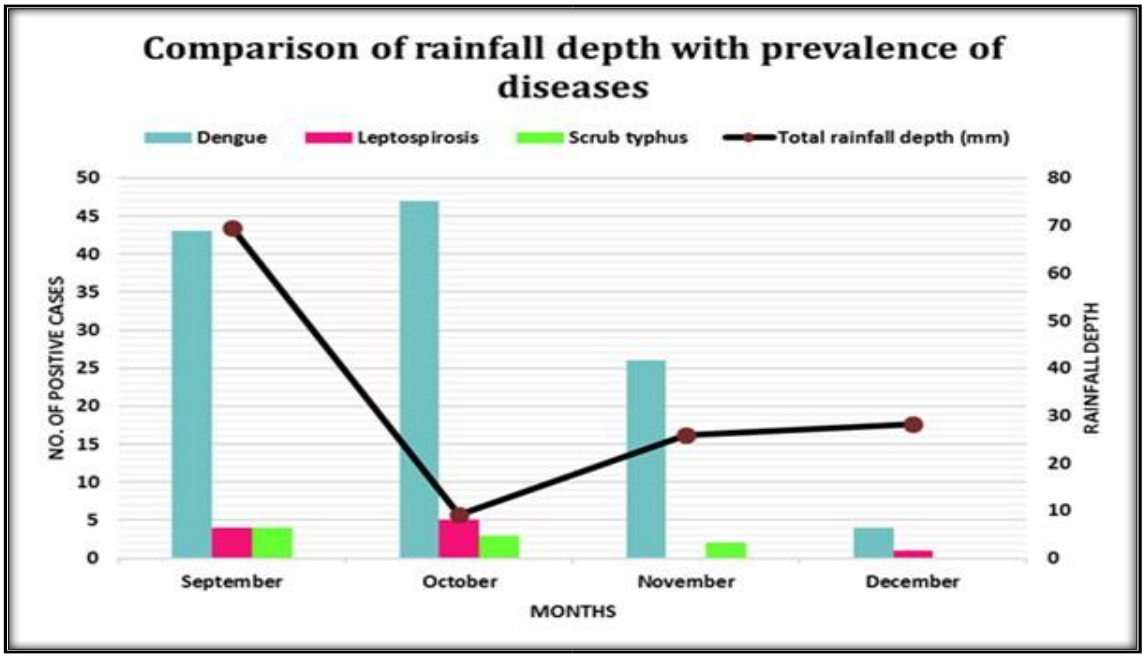

Figure 5: Total rainfall depth recorded in 4 months in different districts covered under the study

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District Patiala recorded the highest number of cases, followed by District Sangrur, District Ludhiana in Punjab [Figure 6]. The maximum number of cases of all three infections have been recorded in the Patiala district.

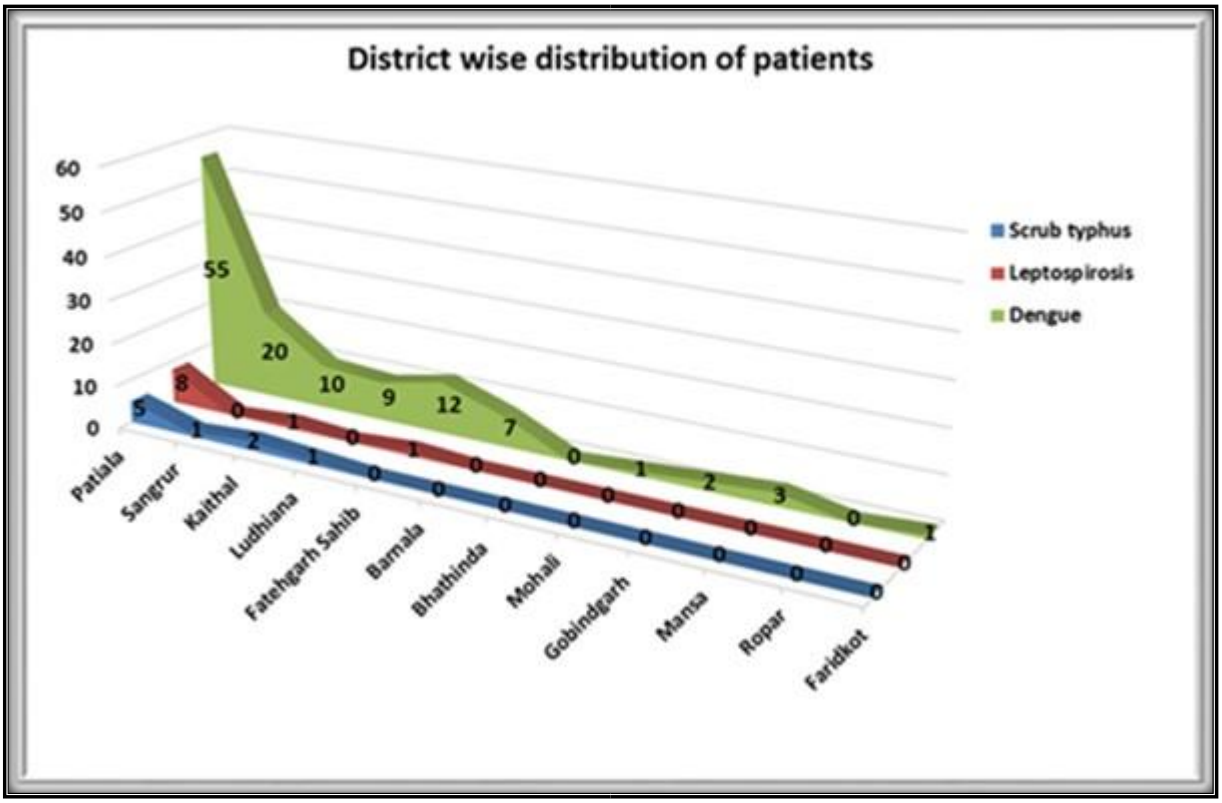

Figure 6: District-wise distribution of Dengue, Leptospirosis, and scrub typhus cases

In the whole study group, the high prevalence was found for dengue cases with no coinfection (90.83%), followed by cases with dengue and leptospirosis co-infection (6.66%) and at last cases with dengue and scrub typhus co-infection (2.50%) [Figure 7].

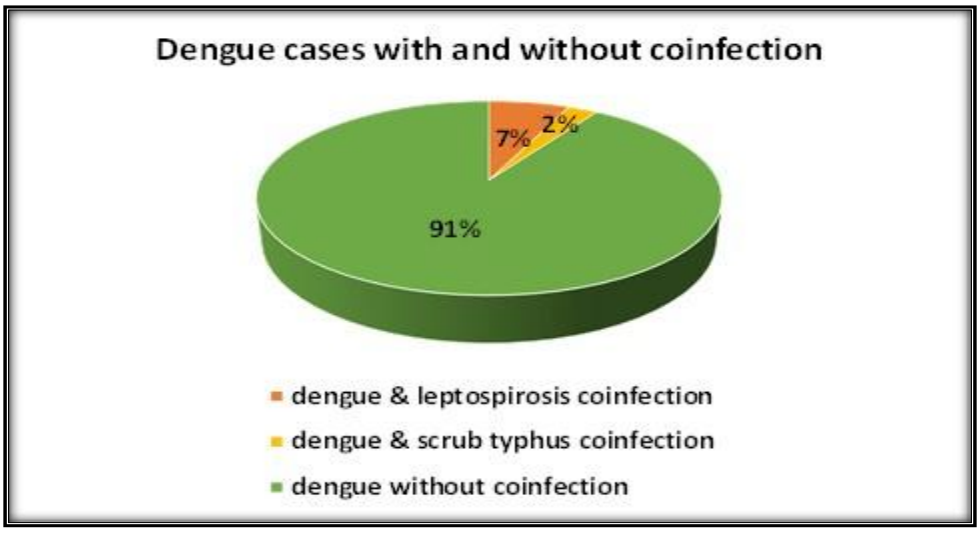

Figure 7: Distribution of dengue cases with and without co-infection

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DISCUSSION

The results of the present study exhibit the current prerequisite of effectively diagnosing the underlying cause of acute febrile illness (AFI) and fever in our country. Fever is a nonspecific manifestation of many infections. Dengue fever is the most common fundamental cause of AFI in our region. However, other infections such as leptospirosis and scrub typhus can also be prevalent in the same season. The overlapping of these zoonotic diseases, leptospirosis and scrub typhus with dengue infection, sometimes causes underreporting of such cases. Overlooking of co-infection of these diseases may lead to inadequate treatment and increased mortality rate among patients. So, there is a need to properly look for such coinfections prevalent in the same season so that timely administration of antibiotic therapy may save lives.[15]

Dengue has been recognized as an endemic disease in India for over two centuries. Global studies have anticipated that ecological and climatic factors encourage the seasonal incidence of dengue infection.[16–18] The dengue incidence was correlated to rainfall data of 2019. There was high rainfall recorded in the month of September- December 2019. Studies on the comparative incidence and dissemination of dengue have shown that maximum A. aegypti larval indices were found during the monsoon and post-monsoon period.[19–21] The highest cases of these diseases were found positive in the post-monsoon season. The stagnant water in low-lying areas during post-monsoon season acts as the breeding ground for mosquitoes. So there is a high surge in dengue cases in these months. In our study, the highest number of dengue cases were recorded in October, i.e., 47 cases (39.1%) and September 43 cases (35.8%), followed by November and 26 cases (21.6%) and December, 4 cases (3.3%). Our results are in tune with those of.[15] They observed no positive dengue cases in the premonsoon period. During the monsoon period, i.e., in August, only 3 cases (0.34%), and in September, 68 cases (7.6%) were confirmed to be dengue positive. The highest number of positive dengue cases were reported during the post-monsoon period, with a maximum number of instances, i.e., (65.3%) in October, followed by those in November and December. When the statistical analysis was performed, the correlation between rainfall and the prevalence of these diseases was not found statistically significant. Salam N also reported a very weak correlation between rainfall and dengue incidence.[22]

Table 1: Statistical analysis of the results

S.no.	Parameters	Statistical Analysis	P value	Significant
1.	Age and Disease prevalence	One way ANOVA followed by Tukey's multiple comparison test	0.0047	YES
2.	Dengue and Co-infection	One way ANOVA followed by Tukey's 1multiple comparison test	<0.0001	YES
3.	Months and Disease	One way ANOVA followed by Tukey's multiple comparison test	0.0109	YES
4.	Gender and Disease Prevalence	Chi-square test	0.0067	YES
5.	Disease and District			

a.	Dengue vs Leptospirosis and District	Two way ANOVA	<0.05	YES
b.	Dengue vs Scrub typhus and District	Two way ANOVA	<0.01	YES
c.	Leptospirosis vs Scrub typhus	Two way ANOVA	>0.05	NO
6.	Rainfall vs Diseases	Correlation	>0.1	NO

In males and females, we found that males are at high risk of acquiring dengue infection than females. There is a significant difference between the prevalence of dengue infection among males and females (P<0.005). This variation may be due to socio-cultural differences where males are more exposed to outdoor activities, and their bodies are less covered than females.[23,24] Other studies have also shown a pattern of males being more affected than females.[25] When different age groups were examined for dengue prevalence, there was a significant difference in the number of dengue patients in each age group (P<0.05). The age group of (21-40) yearswas found to be the most infected with dengue, while the age group of (61-80) years had the fewest instances. Haroon et al. 2018 have also reported the highest prevalence of dengue in the age group of (21-40).[26] There is statistically significant variation between the number of patients with dengue as compared to other diseases in various age groups (P<0.05). The engagement of this age group of people in occupational and recreational activities may be considered as the reason for this prevalence.

Besides dengue, other infections such as leptospirosis and scrub typhus prevalent in this season are also responsible for acute febrile illness. So all the samples tested for dengue infection were also tested for leptospirosis and scrub typhus considering the chances of coinfection or any of these zoonotic diseases responsible for AFI. In leptospirosis, early diagnosis is essential to differentiate it from other causes of acute febrile illness. This is essential to start timely antibiotic therapy.[27] Detection of leptospirosis by IgM ELISA provides positive results in the initial phase of the disease, thus helping in suitable treatment. Agriculture is an essential occupation in this region of Punjab. Misuse of fertilizers makes the pH of water and soil alkaline, creating favorable conditions for the persistence of Leptospira, further encouraging its transmission. There is a strong impact of rice cultivation as a risk factor for leptospirosis.[28] The paddy field workers usually stand for many hours in the water to implant rice in the mud field. That water may be contaminated with the urine of leptospirosis-infected rodents. This exposure of several hours is a vital risk factor among the exposed individuals for the development of leptospirosis.[29] Other studies have also reported that the farmers of paddy cultivation are more significantly infected with leptospirosis than other residents of the same area.[30] Contact with animals and bathing in contaminated community ponds is a part of rural life across north India.[31] This might also pose as a significant element responsible for leptospirosis transmission.

In our study, the highest leptospirosis cases were found in the month of October (5 cases), followed by September (4 cases) and December (1 case). There were no leptospirosis cases in the month of November. There was no statistically significant variation in the gender-wise distribution of leptospirosis cases in our study (P<0.005). 3.6% male population and 3.7% female population were positive for IgM antibodies against leptospirosis. The highest number of leptospirosis patients were found in the age group of 21-60 years. Mainly this age group is involved in farming and other professional activities.

Although being a geographically confined rural tropical infection of hilly areas in India, scrub typhus is now not uncommon to present in tertiary care hospitals in Punjab as visitors

from Punjab often travel to Himachal Pradesh in this season. Recreational activities like trekking in vegetation covered hilly areas which might carry scrub typhus mites may lead to scrub typhus infection.[32]

In the present study, scrub typhus cases were also seen in the post-monsoon season. Collection of rainwater in small pools post-monsoon leads to an increase in scrub vegetation cover responsible for the multiplication of scrub typhus mites. Scrub typhus was reported highest in the month of September (4 cases) followed by October (3 cases) and November (2 cases), but there were no scrub typhus cases in the month of December. There is a high incidence of scrub typhus infections too in the post-monsoon season. This is probably due to an increase in scrub vegetation, leading to an increase in scrub typhus mite population as these bushes act as a habitat for chiggers. Gupta et al. 2016have reported most of the scrub typhus cases in the months of September to November.[33] Similarly, Vivekanandan et al. 2010 have reported a maximum number of scrub typhus cases in cooler months after rainfall, i.e., between September and April.[34] So all these diseases are most commonly prevalent in the post-monsoon season as surplus water logging due to rainfall provides breeding grounds for vectors of these diseases. 3.6% male population and 2.9% female population were positive for IgM antibodies against scrub typhus. The same age group trend as observed in the case of leptospirosis was observed in the scrub typhus patients, i.e., 21-60 years age group reported the highest number of cases.

When district wise distribution of all infections was surveyed, the highest number of dengue patients, leptospirosis, and scrub typhus cases were recorded in Patiala district. The variation in prevalence of all infections in this district was found to be statistically significant($P<0.01$). There is no significant variation in the number of cases of different diseases in all other districts.

According to a case report by Sapkota et al. 2017, 4.1 % of patients presenting with acute febrile illness were positive for leptospirosis, and 3.2% were positive for scrub typhus.[35] In diagnostic surveillance carried out by Rao et al. 2019, 25.3% of patients tested positive for dengue, and 3.5% of patients tested positive for scrub typhus.[36] In an open cohort study carried out in a hospital, scrub typhus was diagnosed in 22.5% of patients, dengue fever in 7.8 % of patients, and leptospirosis in 0.6 % of patients.[37] Other studies worldwide have reported co-infection ranging from 1.3% to 17.5 %.[38,39]

The difference between leptospirosis and scrub typhus patients is not statistically significant ($P<0.05$). Our study found that the number of patients carrying co-infection or without coinfection was variable. 7% of patients had a co-infection of dengue and leptospirosis, and 2.5% of patients carried co-infection of dengueand scrub typhus. The remaining 91% carried onlydengue infection. This difference is highly significant ($P<0.0001$). In a study conducted by Sachu et al. 2018, dengue and leptospirosis co-infection was seen in 3.4% of samples.[38] According to Watt et al. 2003, leucocytosis and thrombocytopenia favor the prevalence of scrub typhus infection, whereas leucopenia and bleeding gums indicate the presence of dengue fever.[40] The whole picture hinted towards the existence of co-infection of dengue and scrub typhus. They also conveyed that the patients with co-infection had lower hemoglobin levels, but normal leucocyte count compared to those with only dengue or scrub typhus infection.

CONCLUSION

To conclude, patients in tropical countries presenting in the post-monsoon season with acute febrile illness not responding to appropriate and adequate therapy aimed for a suspected tropical infection such as dengue should be evaluated for concurrent infections with other

microorganisms. This possibility of co-infection should be thought of timely enough to decrease morbidity as well as mortality. The clinical features of these ailments are nonspecific and so coinciding that it is very tedious to accomplish a differential diagnosis. Serological methods like ELISA hereby prove the specific and quick method to differentiate between the diseases considered in the present study.

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