Comparative Study On Sero Prevalence Of Leptospira–IgM Among Critically Ill Patients And A Community Where Ideal Environment For Leptospirosis Occur : A Pilot Study In A Medical College & Hospital.

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Abstract:

Introduction: Leptospirosis is an emerging widespread zoonosis, caused by leptospira species. This type of diseases mostly occur in tropical, subtropical region and is a major public health problem with outcomes ranging from subclinical infections to fatal disease form known as Weil's syndrome. The causative agent of weil's disease is named as L. icterohaemorrhagia was isolated in 1915 by Inada.

Method: In this study, total 57 blood samples were collected from hospitalised patients who were suspected as leptospira infected as test samples and 10 samples were collected from community as control and diagnosed for leptospira specific IgM by indirect ELISA.

Result: Out of 47 samples, 5 samples (10.635) were found to be positive and patients with positive leptospira IgM were of working age group having higher chsnce of exposure to the pathogen. On the other hand, out of 10 controls, only 1 control (10%) was found to be positive.

Conclusion: From the study, it can be deduced that leptospirosis spreads from soil or water infected with animal urine that carries the pathogen. All the positive test samples were from patients who mainly reside outside the town area and may have direct contact with such infection source for their occupational purpose. Leptospira carried by the positive control was actually acquired from a trip to Sundarban. It also can be concluded by comparing with reports from others that in this area, rate of leptospirosis is low compared to others even in monsoon.

Key words: Leptospirosis, Zoonosis, Weil's disease, IgM, ELISA

Introduction:

In 1886 leptospirosis was first described by Adolf Weil & leptospirosis causative agent Leptospira was first observed in 1907 from a post mortem renal tissue slice[1]. Leptospirosis is an emerging widespread zoonosis, caused by leptospira species. This type of diseases mostly occur in tropical, subtropical region and is a major public health problem with outcomes ranging from subclinical infections to fatal pulmonary haemorrhage and flu-like illness to a severe disease form known as Weil's syndrome. The causative agent of weil's disease is named as L. icterohaemorrhagia was isolated in 1915 by Inada. Severe disease of leptospirosis includes jaundice, acute renal , intravascular disease and hepatic failure, pulmonary distress, which may result in death[2]. Leptospires are divided into pathogenic, non-pathogenic, and intermediate /opportunistic species based on DNA hybridization studies. The current study in genomic based classification indicates that there are at least 19 species which are divided into 13 pathogenic species and 6 saprophytic species[3,4], identified through DNA hybridization analysis [5,6]. Among these 19 species 7 species are the main agents of leptospirosis, those are : *L. interrogans*, *L. borgpetersenii*, *L. santarosai*, *L. noguchii*, *L. weilli*, *L.kirschneri* and *L. alexanderi* [7]. Under the new classification all recognized species are further subdivided into 24 serogroups and more than 200 serovars based on the surface lipopolysaccharide (LPS)[3,8]. *Leptospira interrogans* is very thin, flexible, tightly coiled, obligate aerobic spirochaete characterized by a unique flexuous type of motility with a single axial filament and hooked ends. An important feature of the spirochetes is the location of the flagella, two endoflagella with their free ends towards the middle of the bacteria lie in the periplasmic space between the cell wall and the outer membrane. The motility of bacteria with external flagella is hindered in viscous solutions, but that of spirochetes is enhanced and it is theorized that this kind of flagella is responsible for the ability of spirochaetes to penetrate and invade host tissue. They do not stain well with conventional dyes, and resembles gram negative bacteria because of the Lipopolysaccharide membrane. They can only utilize Long Chain Fatty Acids as their sole carbon and energy source. Optimal growth temperature of pathogenic species in culture is 28°- 30°C .

They grow very slowly with a generation time of about 20 hours, colonies are visible after 3-4 weeks on solid medium.

Leptospirosis is a potentially fatal zoonosis that is mostly occurs in heavy rainfall, flooded and poor sanitisation areas . In west Bengal specially in Kolkata, there is high average rainfall with water holding capacity of soil [9]. As rodents like rats, livestock and pets are reservoir for leptospires, their urine containing leptospires can contaminate the water by which humans get infected either by direct or indirect exposure to this contaminated water. Rats are main hosts of serovar Icterohaemorrhagiae, cattle of Hardjo and Pomona, pigs of Pomona or Tarrossovi, and dogs of Canicola[10]. On the other hand people living in urban slum encirclement with inadequate sanitation are at high risk of rat exposure and leptospirosis. Farmers, Sewage workers, Miners, Veterinarians and individuals who are involved in Water sports, Gardening, Ecotourism are at high risk for leptospirosis [11,33,61]. These occupations involve activities likely to result in exposure of wounds, cuts and to soil and water contaminated with the urine containing leptospire of rodents and animals from which workers get infected .All these above points are crucial reasons for the cases of leptospirosis in Kolkata.

The pathogenesis of Leptospirosis is not clearly known till now. Leptospires generally gain entry through small areas of damage on the skin, the conjunctiva or via mucous membranes and abrasion. They enter and spread to the whole body system and infect kidney, liver, heart and even Central Nervous System and may result rapidly from an apparently mild illness to severe condition such as pulmonary haemorrhage, jaundice, acute kidney injury and meningo encephalitis. After reaching the number of leptospires in the blood and tissues at critical level, lesions develop due to the action of undefined leptospiral toxin(s) may known as endotoxin or toxic cellular components and consequent symptoms appear. Endotoxin activity has been observeded in several serovars of leptospires [12,13] Severe cases of leptospirosis should be treated with high doses of intravenous penicillin, less severe cases can be treated with oral antibiotics such as amoxicillin, ampicillin[14]. Third-generation cephalosporins antibiotics also appear to be effective against leptospirosis[15]. The clinical diagnosis of leptospirosis are often nonspecific, that’s why a timely and accurate laboratory diagnosis is essential to diagnose leptospirosis cases. Several

types of method are used to diagnose leptospirosis such as: polymerase chain reaction (PCR)[16], Microsopic agglutination test(MAT), ELISA.

MAT, the reference serological test is considered as the 'gold standard' of serodiagnosis although with some limitations[17]. MAT requires technical expertise, can be done only in reference laboratories that maintain live *Leptospira* strains, and is the best interpreted with both acute and convalescent sera. Additionally, a live panel of leptospires require in MAT, is a laboratory biohazard[18]. Other limitations include a limited sensitivity during the early phases of illness(19), inter-laboratory variation due to subjective interpretation of agglutination and difficulty in standardisation[19,20].

ELISA is the simplest tool for the diagnose leptospirosis .Leptospira specific IgM antibody may be observed after 4 to 5 days of the onset of symptoms[21]. Samples can be screened for anti-leptospira IgM by ELISA. Positive ELISA is confirmed by using MAT. One study (Roy Sagnik et al) has been showed that the ELISA tests are the most readily applicable for the rapid detection and diagnosis of leptospirosis[9].

Mass immunization applying in people to prevent of this disease. Awareness of Leptospirosis through the advice of doctor, employers and general public will help to develop safer practices during recreational pursuits. Vaccination of human is beneficial method ,where they are usually associated with animal sources though no universally accepted vaccine is available for human. Personal hygiene, Personal protective equipment, proper water treatment and most importantly control of rodents are some excellent way to prevent leptospirosis. The number of human cases over leptospirosis in worldwide is not known briefly. In October, 1995, it was reported that in rural Nicaragua ,epidemic hemorrhagic fever, caused by leptospira not by jaundice or renal manifestation [22]. According to the current study it has been seen that incidents range from approximately 0.1-1/100000/year in temperate regions to 10-1000/100000 in the humid tropic region. Unlike leptospirosis mild cases can not be diagnosed. The mortality rate of leptospirosis is high, ranging from 2.5%to 16.45%. The mortality rate can be up to 56% at the age over 50 [9]. ln 2011 reports from the Southern part of Gujarat revealed that 130 people were died within a span of two months due to only leptospirosis. In October 2012 one study reported 16 deaths were observed in Surat and Valsad districts of Gujarat

due to leptospirosis [23] .The true statement of human leptospirosis in West Bengal state is not clearly known because lack of proper diagnostic techniques. In the year of 2013 -2016 one report from Kolkata showed that out of total 1527 patients 562 (36.8%) were diagnosed for leptospirosis. Out of these 562 diagnosed patients male patient was 410 (72.9%) and female was 152 (27.1%) and all the samples gave positive result in ELISA test [24]. Most of the people of this part of the planet suffering from infectious jaundice are sometimes confused with a viral hepatitis, but, many of these cases might be due to Leptospira infection.

The aim of this study is to compare among the rate of the infected patients those who were suspected to leptospirosis, already admitted into hospital and, citizens (as a control) from a community where an ideal environmental condition for leptospirosis occur. We were focused mainly in the monsoon time (June-August) to get better results from the surrounding area.

Review of literature:

Leptospirosis is an emerging widespread zoonosis, caused by pathogenic spirochaetes of leptospira microorganisms [10, 11, 25, 34]. Under the new classification all recognized species of leptospira are further subdivided into 24 serogroups and more than 200 serovars based on the surface lipopolysaccharide (LPS) & DNA hybridization [3,8,10].

The genomic based classification of leptospirosis indicates that there are at least 19 species which are divided into 13 pathogenic species and 6 saprophytic species, identified through DNA hybridization analysis [3,4,5,6].

Among these 19 species 7 species are the main agents of leptospirosis, those are: *L. interrogans*, *L. borgpetersenii*, *L. santarosai*, *L. noguchii*, *L. weilli*, *L. kirschneri* and *L. alexanderi* [7].

Saprophytes are supposed not to cause disease. They are often found in cultures from clinical materials, but the significance of their presence is uncertain. Saprophytic species of *Leptospira* include *L. biflexa*, *L. meyeri*, *L. yanagawae* (genomospecies 5), *L. kmetyi*, *L. vanthielii*

(genomospecies4), and *L. wolbachii*, and contain more than 60 serovars [12,26,27].

Pathogenic leptospires can be maintained in nature in the renal tubules of animals and saprophytic leptospires in many types of wet or humid environments.

A special characteritics of the spirochetes is the location of the flagella, two endoflagella with their free ends towards the middle of the bacteria lie in the periplasmic space between the cell wall and the outer membrane. The motility of bacteria with external flagella is hindered in viscous solutions, but that of spirochetes is enhanced and it is theorized that this kind of flagella is responsible for the ability of spirochaetes to penetrate and invade host tissue[12].

Rats are main hosts of serovar Icterohaemorrhagiae, cattle of Hardjo and Pomona, pigs of Pomona or Tarrossovi. L canicola is transmitted by dogs[10,28,29].

The disease typically occurs as an epidemic lasting a few months during the monsoon season. The transmission cycle involves interaction between one or more animal hosts harboring *Leptospires*, an environment favorable for its survival, and human beings.[31,32]

Farmers, Sewage workers, Miners, Veterinarians and individuals who are involved in Water sports, Gardening, Ecotourism are at high risk for leptospirosis.[11,31,33,61].

It has been reported that the northern provinces of Iran, particularly Guilan and Mazandaran, are ideal areas for transmission of *Leptospira* due to their humid climate, high population densities, and rural agricultural (mostly
rice farming) and fishing activities[35].

Leptospirosis epidemics in tropical countries are often related to heavy rainfall and flooding. The Indian state of Kerala has witnessed post-monsoon epidemics of leptospirosis in recent years.[36,37,38]

The microagglutination test (MAT) is the most common serological test for antibody detection, but cross-reaction between serovars is a known limitation and can affect the interpretation of results[40].

Leptospires generally gain entry through small areas of damage on the skin, the conjunctiva or via mucous membranes and abrasion. They enter and spread to the whole body system and infect kidney, liver, heart and even Central Nervous System and may result rapidly from an apparently mild illness to severe condition such as pulmonary haemorrhage, jaundice, acute kidney injury[40,41]

In vitro studies have shown that pathogenic leptospires can evade host defense mechanisms by surviving within macrophages, delaying phagosome maturation , and can resist to reactive oxygen species[41,42,43].

However, it is estimated that 10 or more per100,000 people are afected with this disease each year in tropical climates[44].

Common complications of the disease include renal failure, respiratory failure, neuroleptospirosis, and Disseminated Intravascular Coagulation. Clinical features include headache, fever, myalgia, jaundice, conjunctival sufusion, bleeding tendencies, oliguria, and pulmonary manifestations like cough, breathlessness, and hemoptysis . The mild, anicteric form of the disease is more common and presents with nonspecific[44,45,46]

Leptospirosis is highly prevalent in Asia Pacifc Region and outbreaks in developing countries are most frequently related to normal daily activities, overcrowding, poor sanitation, and climactic condition[47,48].

The leptospiral life cycle involves shedding in the urine, persistence in the ambient environment, acquisition of a new host, and hematogenous dissemination to the kidneys through the glomerulus or peritubular capillaries. Once leptospires gain access to the renal tubular lumen of the kidney, they colonize the brush border of the proximal renal tubular epithelium, from which urinary shedding can persist for long periods of time without significant ill effects on the reservoir host. For this reason, leptospiral infection of the reservoir host can be considered a commensal relationship[11]

At critical level of leptospires number in the blood and tissues, lesions may develop due to the action of undefined leptospiral toxin(s) may known as endotoxin or toxic cellular components and consequent symptoms

appear. Endotoxin activity has been observeded in several serovars of leptospires [12,13]

Several leptospiral recombinant antigens were evaluated as a replacement for the inactivated (bacterin) vaccine; however, success has been limited. A prospective vaccine candidate is likely to be a surface-related protein that can stimulate the host immune response to clear leptospires from blood
and organs.[49]

The variation in canine leptospirosis risk in specific counties and regions of
the USA appears to be mainly influenced by environmental and land use factors.[50]

Overall Weil's syndrome has a mortality rate of 5% to 10%. Important causes of death include renal failure, cardiopulmonary failure and widespread haemorrhage[51,52]. The diagnosis of leptospirosis is difficult because the disease's numerous manifestations can mimic other tropical infections or other nonspecific febrile illnesses, as well as noninfectious diseases[53].

Leshem et al. reviewed 48 cases of travel-related leptospirosis seen in Israel between 2002 and 2008, while Van Crevel et al. reported 32 such cases
in the Netherlands between 1987 and 1991.one study was reported that 15 cases of travel related leptospirosis. Among these 15 patients 14travelars (patients) who were suffered in leptospirosis were men, whose mean age was 34 years[54].

An increase in the incidence of leptospirosis in France and the Czech Republic was attributed to flooding events in 1997 and 2002[7,55] In the Asia-Pacific region, leptospirosis is determined as a waterborne disease; recent outbreaks in Indonesia (2003), India (2005), Sri Lanka (2008) and the Philippines (2009) occurred after major urban flooding, dispersing leptospires in contaminated waters[56,7,57].

In October, 1995, it was reported that in rural Nicaragua ,epidemic hemorrhagic fever, caused by leptospira not by jaundice or renal manifestation[22].

Out of these 562 diagnosed patients male patient was 410 (72.9%) and female was 152 (27.1%) and all the samples gave positive result in ELISA test [24]. Urban leptospirosis situation is expected to become more urgent as the world's urban slum population is estimated to dramatically increase in the next 20 years [United Nations, 2003][61].

In November 1961, An outbreak of Leptospirosis occurred in november 1961 among 186 US Army Troops in the canal zone during exercise in jungle10 to 13 days earlier.[22,58] A high prevalence of leptospiral antibodies in humans was reported from Somalia in 1982 [59]. Another survey in 1987 in Italy showed a prevalence in rural areas of 11.34%, while it was 3.08% in urban areas of central Italy [60].

Materials and Methods:

Our study was conducted during two months (July-August) in KPC Medical college & Hospital.

Sample selection:

We visited to the male medicine wards(1, 2, 3, 4) and Intensive Care Unit(ICU)s of KPC Medical college and hospital to recognise those patients, suspected for leptospirosis were evaluated on the basis of their case history, epidemiological risk factors, laboratory findings and clinical findings as per criterion (Table 1). Samples were collected from recognised patients which had been used as test sample. Several samples were also collected from community include urban slum area or insalubrious encirclement which is detrimental for health, were taken as a control. We got 47 of test sample and 10 of control . Samples were taken into glass vial very carefully.

Laboratory procedure:

Serum was separated from blood sample by using centrifing the sample at 2000RPM for 15-20minutes at room temperature; then serum was taken into another clear eppendorf and stored at -20°C until it was tested. Serum was tested for qualitative detection

Clinical features (A)	Score
Fever	2
Headache	2
Temperature > 39°C	2
Myalgia	4
Conjunctival suffusion	4
Meningism	4
Jaundice	1
Albuminuria/ elevated BUN	2
Epidemiological factors (B)	
Rainfall	5
Contaminated environment	4
Animal contact	1
Laboratory criteria (C)_____	
Culture	Diagnosis
certain ELISA IgM	
15	MSAT
15	MAT- single positive high titer
15	MAT- rising titer (paired sera)
25	

of leptospira specific IgM antibodies using lepto IgM Microlisa test, which is an enzyme immunoassay based on “Indirect ELISA”. Once the assay has started, full procedure was completed without any interruption. Briefly for ELISA, tested serum samples were gently mixed with Rf absorbent in 1:11 dilution in separate tubes (10 µl serum samples + 100 µl Rf absorbent) and incubated for 10 mins in room temp. 25 µl of this mixed sample from each tube were used for the ELISA procedure. 100 µl of sample diluent (Buffer containing protein stabilizers) were added in each antigen coated microwell including the positive and negative control wells; kept it for 30 mins at 37°C. Each wells were carefully washed 5 times with wash buffer solution. 100µl antihuman IgM labelled with horseradish peroxidase were added in each well and incubated for 30 mins at 37°C. Again washed it 5 times; then 100 µl of TMB substrate were added and incubated at RT for 30 mins. 50 µl of 1N sulphuric acid (stop solution) were added. The absorbance was taken at 450 nm/630nm. The reactivity of serum samples were construed on the basis of calculation on of the lepto IgM unit. Lepto IgM unit were derived from calculation process of cut off value as follows:

Cut off value = Mean value of negative control + 0.500

Ratio of sample O.D. = Sample O.D. / Cut off value

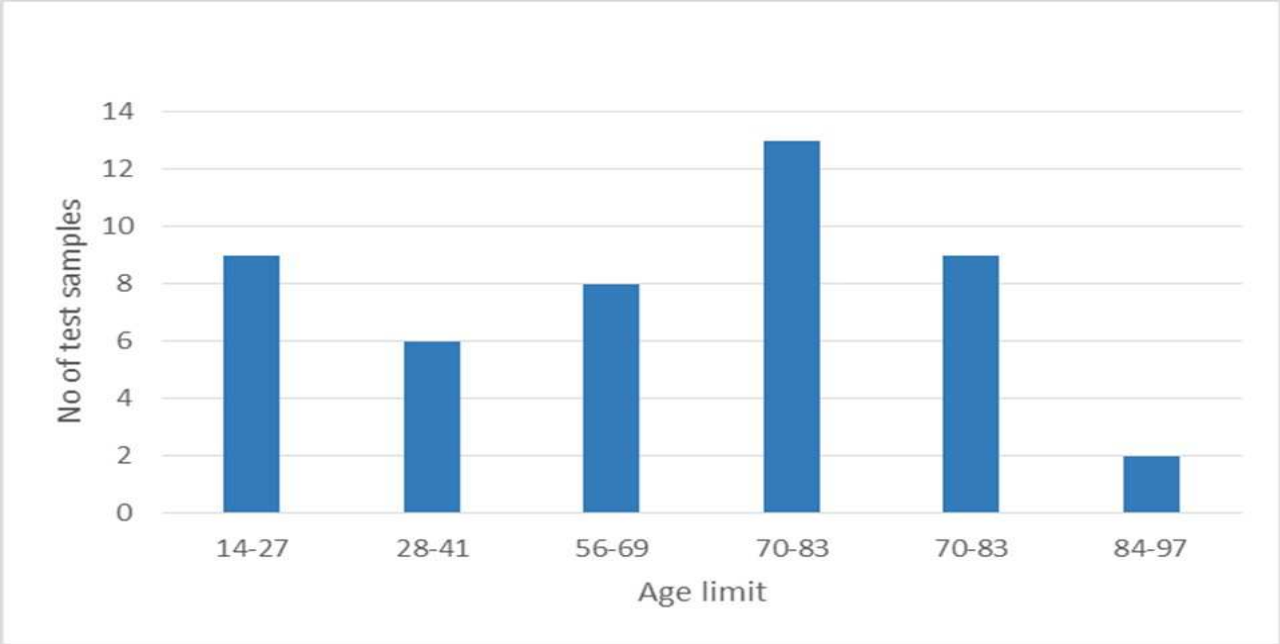
Lepto IgM units = Ratio of sample O.D. x 10

When sample lepto IgM unit is > 11; it was interpreted as a sample is reactive but when the unit is < 9 then it was interpreted as the sample is nonreactive. For sample showing the unit lies between 9 -11 that determined as equivocal result; another blood sample was taken from same person after a period of 10 days.

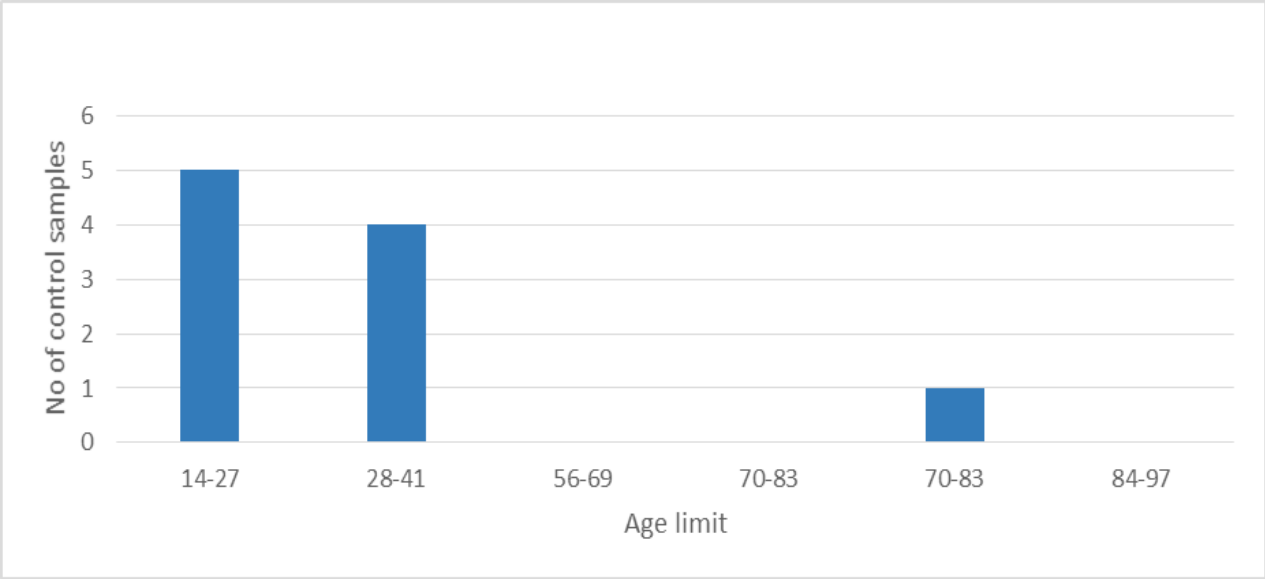
Result:

In this study we tested total 57 blood samples. Among those 57 samples 47 samples were collected from hospitalized patients (test sample) (Table1) and 10 samples (control) were collected from locality and diagnosed thoroughly for leptospirosis (Table 2). Out of 47 samples, 5 samples(10.63%) were found to be positive for IgM antibody against Leptospira (Table3); on the other hand out of 10 samples, only 1 sample (10%) was found to be positive. So that we got totally 6 samples(10.52%) out of 57 samples which were positive for IgM antibody against Leptospira.

Serial No.	Age Limit	No. Of Test Samples
1	14-27	9
2	28-41	6
3	42-55	8
4	56-69	13
5	70-83	9
6	84-97	2



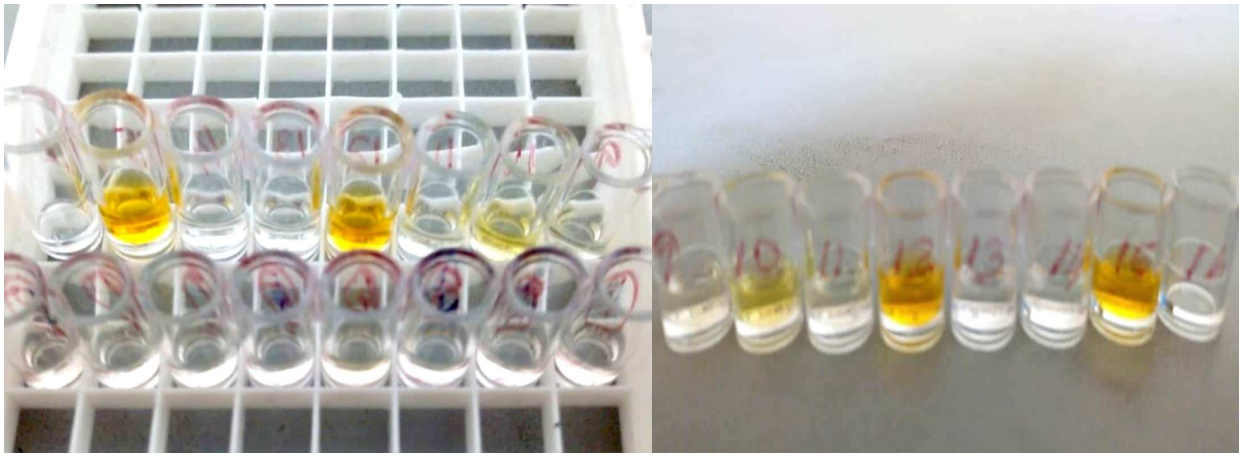
Serial No.	Age Limit	No. Of Control
1	14-27	5
2	28-41	4
3	42-55	0
4	56-69	0
5	70-83	1
6	84-97	0



Serial No.	Age Limit	No. Of +Ve Test Samples
1	14-27	0
2	28-41	0
3	42-55	1
4	56-69	2
5	70-83	1
6	84-97	1

Table 4 shows the distribution of leptospirosis patients according to clinical presentation. The commonest features included fever (69%), headache (70%), nausea(76%). The CRP value, bilirubin values were observed respectively in those patients who were suspected to this disease.

Serial No.	Clinical Findings	No of Test Samples
1	Fever	38
2	Pedal oedema	12
3	Liver Function Test	40
4	Urea	50
5	Creatinine	50
6	CRP value	7
7	Bilirubin	22



Other clinical features were malaise, fatigue, cold, cough, bodyache, distended abdomen, uremia, vomiting, arthritis, pedal edema, diarrhea. Some leptospira cases had bilirubin value below 1mg/dl with in range 0.5-0.9mg/dl but it was found that one case had bilirubin value higher than 1mg/dl. Higher level of urea value that means >20mg/dl were found in 84% leptosiral cases. 85% Patients suffered in renal failure with serum creatine value > 2mg/dl where as the normal range is 0.5-6.8mg/dl, gave positive result for leptospiral case. Among them who were investigated for renal parameters, it was seen that around all of them had elevated urea and

creatinine levels. When the clinical outcome of the leptospirosis patients was analysed, it was observed that all of them recovered without any complications and hence the fatality rate was 0%.

Discussion:

The Study was conducted for the two months of this year extending from July-August. Total 57 blood samples were collected & diagnosed thoroughly for leptospirosis. Among those 57 samples 47 blood samples were collected from hospitalised patients which determined as a test samples; on the other hand, rest of those total samples, 10 blood samples were collected from surrounding areas regarding as a control samples.

Persons of all ages and races are susceptible. Adult men however are more frequently infected because they tend to work in high-risk jobs. The number of cases in a region often fluctuates from year to year due to various factors such as rainfall, flooding and animal infections. The severity of leptospirosis depends on a few factors. Those determinants are risk factors of infecting Leptospira; Infectious inoculum at the time of exposure, Pre-existing immunity due to previous exposure, Hormonal influences, Human host genetics and nutritional factors like malnutrition and alcohol consumption [62]. Leptospirosis infection is biphasic, that is it occurs in two phase. One is the acute or septisemic phase lasting about a weak, followed by immune phase, characterised by antibody production and excretion of pathogen through urine. Most of the complications associated with leptospirosis occurs during the immune phase or from the second week of infection [63]. The mortality rate of leptospirosis is high, ranging from 2.5% to 16.45%. The mortality rate can be up to 56% at the age over 50 [9]. Leptospirosis is a widespread and prevalent zoonotic disease. It occurs in both temperate and tropical regions; the incidence in the tropics is approximately 10 times higher than in temperate regions [64]. A modeling exercise by the World Health Organization's (WHO's) Leptospirosis Burden Epidemiology Group estimated that there were 873,000 cases worldwide annually with 48,600 deaths [65]. ln 2011 reports from the Southern part of Gujarat revealed that 130 people were died within a span of two months due to only leptospirosis. In October 2012 one study reported 16 deaths

were observed in Surat and Valsad districts of Gujarat due to leptospirosis[23] .The true statement of human leptospirosis in West Bengal state is not clearly known because lack of proper diagnostic techniques. In the year of 2013 -2016 one report from Kolkata showed that out of total 1527 patients 562 (36.8%) were diagnosed for leptospirosis. Out of these 562 diagnosed patients male patient was 410 (72.9%) and female was 152 (27.1%) and all the samples gave positive result in ELISA test [24]. Out of 42 persons with jaundice who were evaluated in Calcutta, 10 (23.8%) were found positive for leptospirosis [66]. Our study showed only 6 patients were found to be positive for leptospira out of 57 samples, that means only 10.5% people were infected with the leptospira. A study conducted by Meenakshi Mathur, Anuradha De and Dilip Turbadkar in Lokmanya Tilak Municipal General Hospital, Mumbai during July-September 2005, Out of 942 serum samples, 323 were positive by LeptoTek Dri-dot/Leptocheck, with an overall seropositivity of 34.3%. This comprised of 73.4% adults (176 males and 61 females) and 26.6% children (61 boys and 25 girls). The adults to children ratio was 2.76:1 and males to females ratio was 2.76:1 [67]. In Andamans 544 cases were reported during 2000-04 by disease surveillance system. With the highest incidence there were total of 93 deaths in 2002. 58 cases of confirmed leptospirosis were admitted in 2005 and 14 patients died [Case Fatality Rate (CFR)-24.1%]. The reason of death of a major number was due to pulmonary hemorrhage that occurred within 48 hours [68]. 322 of 611 sera samples in 2004, from different high risk populations were positive giving an overall sero prevalence of 52.7% [69].

Due to this endemic disease in South Gujrat, in the year 2005, 392 cases and 81 deaths from various districts were reported. 310 males and 82 females were mostly in the age group of 26-45 years were infected [70]. Common complications include Jaundice, renal failure and haemorrhagic pneumonitis. Based on extensive studies conducted in Gujarat, it was highlighted that agro-climatic conditions for south Gujarat favour endemicity for leptospirosis because of heavy rainfall, clay soil and high water table [68].

In Maharashtra Leptospirosis has been reported regularly since 1998 [68, 71, 72]. In 2005 mainly due to large outbreak, 2355 cases and 167 deaths were reported, during the post monsoon floods. Haemorrhagic

pneumonitis occurred in 35.1% out of 74 cases. Of the 11 deaths (14.8%), 9 were due to pulmonary haemorrhage [73]. In ICU admission study, 60 out of 834 (7.2%) of cases were due to leptospirosis. Mortality rate of leptospirosis was 52% and 95% of these patients were ventilated due to respiratory failure [74]. During the examination of our total 57 patients no any deaths were observed.

Leptospirosis is endemic in many areas of kerala. In a study of 976 confirmed cases of leptospirosis, observed mortality rate was 5.32% and the identified serogroups were Autumnalis, Australis and Icterohemorrhagiae [75]. While studying 282 cases of leptospirosis from Calicut, the common complications were hepatic (69.8%), renal (56.3%) involvement and thrombocytopenia (65.8%) [76]. 900 cases treated over 10 years, Jaundice (80%), renal failure (59%), hypotension (20%) were the common complications noted in a study of leptospirosis from kottayam. Diseased persons were professionally agricultural workers, fishermen and oyster shell catchers (82%). 74%, with a male/female ratio of 7:1 were seen during the monsoon months [68].

Since 1980's, leptospirosis has been reported from Chennai [68, 77]. There has been a dramatic increase in the number of leptospirosis cases and 2765 cases were reported during 2006. During 1987 to 1991 at the General Hospital, Chennai, there were around 159 cases of leptospirosis. Among them, 108 were males and 40.1 year is the mean average. 136 (85%) had jaundice and 120 (75%) had renal failure. 70 patients were dialyzed and 25 patients died (15.6 %) [78]. In the recent past, acute renal failure due to leptospirosis has significantly declined from 31% in 1987–91 to 7.5 % in 1995-2004 at general hospital Chennai [79]. Leptospirosis outbreaks have been reported from 15 districts of Karnataka. During the year 2004, 152 cases and 11 deaths were reported and during 2005, 224 cases and 19 deaths were reported [68]. In study of 733 patients suspected of leptospirosis, 84 (11.45%) were found positive by ELISA. The important complications noted were hepatic (65%) and renal failure (63 %) [80]. During making our study which is conducted in Kolkata for two months (mainly rainy season) we found two patients who were admitted in ICU due to their serious condition, may be fever or renal failure; but no deaths were reported.

After the cyclone during the October-November 1999, 142 patients with febrile illness and haemorrhagic manifestations were evaluated in Orissa. 28 (19.2%) evidence of leptospirosis was confirmed by MAT. 6 were positive by culture/PCR [81]. 143 people suspected of leptospirosis in a remote village of Mayurbhanj district in north Orissa was evaluated by the Orissa Multi-disease Surveillance System (OMDSS) during the period June-July 2002 and the attack rate was 5.95%. There was exposure to infected water in a canal which was probably the source of infection [82].

Data from Andhra Pradesh, Uttar Pradesh and Delhi are becoming available. Evaluation of acute febrile patients in Uttar Pradesh revealed that 7% (25/346) had leptospirosis. 17 of the 25 patients had jaundice [83]. In a study of 55 cases of leptospirosis in Hyderabad, 52% had renal failure and jaundice occurred in 42% [84]. In Delhi, 75 patients with symptoms of leptospirosis were evaluated, 32 were found positive for leptospirosis and 5 died [85]. 180 febrile patients from urban slums of Delhi were evaluated and 27 (15%) were positive for leptospirosis [86]. In a study of 33 icteric patients from Puducherry, 22 had altered sensorium and 20 had multiorgan failure and thrombocytopenia. 13 patients died (39.3%) [87].

Conclusion:

Through this project work, we can depict this conclusion that we have gotten 6 leptospira positive patient by testing of total 57 samples. Among the 6 Leptospira positive patients, 4 are of working age group, that’s why they have a great chance to get exposed to the Leptospirosis pathogen may be through their profession. One of them is 92 years old, she had already been admitted in to ICU, because she may had with an immune compromised state considering her age. And the last one, detected Leptospira IgM positive is of 21 years old, student from the institution from which the whole study is conducted, had acquired infection from a recent visit to Sundarban area, which has been known from his case history file. All of these 5 patients exclude 1 patient were from rural areas beside Kolkata and possess an ideal environment for the acquisition of this disease. Mainly leptospirosis occurs in the rainy season; poor land areas including urban slum areas, water logged areas are highly responsible for outbreak of this disease due to heavy raining. Treatment of those affected

patients with proper antibiotics as soon as diagnosis of Leptospirosis & control of rodents is important because they are the main host to spread this disease. Presently, in the flood prone and severe water logging areas in our state an extra cautious is necessary too.

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Conflict of interest statement:

We declare that we don't have any conflict of interest.

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