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"Epidemiology of Human Leptospirosis in HIV Patients Attending Anti-Retro Viral Treatment in Public Hospitals and Clinics in Kabwe Urban"

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Abstract

A cross sectional study was carried out to assess the epidemiology of leptospirosis in 282 participants, 150 were Human immune deficiency virus (HIV) positive patients and 132 non-HIV patients (controls) attending ant retro viral treatment (ART) and outpatient department in public hospitals and clinics in Kabwe, Zambia urban district respectively. Demographic, disease history, co-morbidities and concomitant medication history was captured using a structured 10-point close ended questionnaire. Plasma was tested for the presence of leptospirosis using the enzyme linked immune sorbent assay (ELISA) as screening test and the dark field microscopy technique (DFM) as confirmatory test to determine disease distribution in the population. Plasma found positive with ELISA and leptospires detected by dark field microscopy were considered to have leptospirosis. Results revealed that 50 out of the 150 HIV positive participants were positive for leptospirosis (33%). This was significantly higher (P=0.002) than in the control group, where only 15 out of 132 participants were found to have leptospirosis (11%). 5 tests were discordant as they gave positive results with ELSA yet leptospires were not detected by the dark field microscopy. The leptospirosis confirmatory test was found to have sensitivity of 94%, specificity of 97%, PPV 98%, NPV 92% and efficiency of 95% which were significant parameters to warrant the adoption of the results and determine the epidemiology of leptospirosis in the areas under study. It was concluded that epidemiology of leptospirosis in Kabwe district is 21% after adjusting for false positives.

Keywords: *leptospirosis, Human immune deficiency virus, dark field microscopy, enzyme linked immune sorbent assay.*

Introduction

Leptospirosis is an infection caused by gram negative, corkscrew-shaped bacteria called Leptospira. Signs and symptoms can range from none to mild such as headaches, muscle pains and fevers to severe with bleeding from the lungs or meningitis (1) if the infection causes the person to turn yellow, have kidney failure and bleeding, it is then known as Weil's disease. (2) If it causes lots of bleeding from the lungs it is known as severe pulmonary hemorrhage syndrome (2).

Up to 13 different genetic types of Leptospira may cause disease in humans. It is transmitted by both wild and domestic animals (3). The most common animals that spread the disease are rodents (5).

The disease results in high morbidity and considerable mortality in areas of high prevalence. It is estimated that around 10,000 cases of severe leptospirosis are hospitalized annually worldwide. The disease is usually endemic in areas with rainy season, humidity, and close human contact with livestock, poor sanitation, and workplace exposure to the organism [2]. In recent years, a new trend in human leptospirosis outbreaks has been observed related to recreational activities among wildlife (a form of tourism that is becoming increasingly popular) and army expeditions, either for training

or for combat-related purposes in similar environments [3]. A systematic literature review of Leptospirosis Burden Epidemiology Reference Group (LERG) reports an estimated global annual incidence of endemic and epidemic human leptospirosis ranging from 5 to 14 cases per 100,000. Endemic human leptospirosis rates have varied by region from 0.5/100,000 in Europe to 95/100,000 in Africa. Based on global data collected by International Leptospirosis Society surveys, the incidence was estimated to be 350,000 – 500,000 severe leptospirosis cases annually [4]. Based on CSO 2016 epidemiological and surveillance report, Zambia's disease burden alone is estimated to be at 20/100,000.

However, data emerging from prospective surveillance studies suggest that most human Leptospiral infections in endemic areas may be mild or asymptomatic. Development of more severe outcomes likely depends on three factors: epidemiological conditions, host susceptibility, and pathogen virulence [5]. Fatality rates reported worldwide vary from 5% to 30%. This epidemiological picture is not reliable because in many areas the occurrence of the disease is not well documented. In addition, mild cases may not be diagnosed as leptospirosis [6]. Case fatality for pulmonary hemorrhagic syndrome and Weil's disease is more than 10% and 70%, respectively [5].

Leptospirosis, especially Cerebral Leptospirosis (CL) is one of the most common central nervous system (CNS) opportunistic infections in HIV infected individuals and also the most common cause of focal deficits in patients with AIDS (21). High Human Immunodeficiency Virus (HIV) prevalence rate in Kabwe district of Zambia provides an inherent risk of CL in individuals that may be co-infected with HIV and Leptospirosis.

The aim of this study was to assess the distribution of Leptospirosis in patients living with HIV and ascertain the necessary health care for the disease.

Methods

Study Area: This study was conducted at the ante retro viral treatment (ART) sites in Kabwe district of Zambia. The area has an altitude range of 20-100 m above sea level, is covered by tropical dry forest vegetation, has an average temperature of 24∘C, and receives between 1000 and 2000 mm3 of rain per year. Kabwe is a state devoted to agriculture and livestock production.

Geographically, Kabwe has some streams, rivers and few mountains, it's largely a plateau. These conditions, create habitat conditions that could influence the ecology and distribution of Leptospira serogroups in the district of Kabwe.

The total number of participants recruited in this study was 282. This included 150 HIV positive patients and 132 non-HIV healthy individuals as controls. Purposive sampling method was used to enroll study participants.

Ethical considerations

Ethics approval was obtained from the University of Zambia Biomedical Research Ethics Committee (Assurance No. FWB00000442, IRC0000223 of IOR0000887). Written permission was obtained from Kabwe Mine Hospital. The information sheet about the study was given to patients, translated in Bemba which is the local language or read out to them in cases where they could not read. Patients were informed about the study and given an option to decide if they did not want to take part in the study.

The purpose of the study was explained to all the study participants and those that declined to participate in the study were not forced, but were assured of their protected privileges and other benefits such as being managed by clinicians as per standard treatment and guidelines. The respondents were interviewed individually in a private room and only 4mls of blood specimen was collected from each participant.

Privacy and confidentiality were maintained by using codes for the patients instead of names in the report and data was kept in a locked cabinet and keys kept by the researcher. Respondents were thus assured of utmost confidentiality. Patient's consent to be included in the study was obtained. Patient's comfort and dignity during and after the procedure was paramount. The well-being and prompt definitive management of the patient was first before the research.

Inclusion and exclusion criteria

This study was limited to male and female adults above the age of 18 years. Participants who had a history of having been transfused within 2 to 3 weeks, cancer, or pregnant, were excluded from the study.

Collection of qualitative data

Information relating to HIV and Leptospirosis was obtained from the prospective participants using a questionnaire. A ten-point Structured interview schedule with close-ended questions was used to collect qualitative data. The interview schedule captured demographic variables, knowledge on Leptospirosis common symptoms and factors associated with these symptoms. The questionnaire was administered in the simple English language and translated into local language (Bemba) for those who did not understand English.

ART testing for HIV

HIV testing by ART centres was done by using the Zambian National algorithm were blood samples were first tested using Determine test kit (Abbott Diagnostic Division, Hoofddrop, Netherlands) as a screening test. This is an in-vitro qualitative immunochromatographic immunoassay for the determination of antibodies to HIV-1 and HIV-2. If the test is positive, then SD bio line (HIV ½. 3.0, standard diagnostic Inc.), was used as a confirmatory test. SD Bio line is an immunochromatographic test for the qualitative detection of antibodies of all isotypes (immunoglobulin G [IgG], IgM, and IgA) specific to HIV-1 and HIV-2 simultaneously in human serum, plasma, or whole blood.

Laboratory testing for leptospira

Leptospira IgG IgM ELISA test method

All samples for Leptospirosis testing were analyzed using max 2 ELISA plates and reagents.

The procedure was performed according to manufacturer's instructions. One hundred microliters of pre-diluted samples were pipetted into the microtiter wells and incubated for 30 minutes at room temperature to allow corresponding specific antibodies present, if any, in the patient's serum or controls to bind to the antigens in the wells. This was followed by a washing step, allowing all unbound antibodies to be removed and therefore not obscure any reaction. One hundred microliters of the conjugate were then added followed by a second incubation step of thirty minutes at room temperature. The plate was then washed 5 times and 100µl of the substrate was added. An incubation period of 15 minutes at room temperature was then followed by addition of 100µl of the stop solution.

Results were interpreted according to the manufacturer's instructions. Negative and positive controls were kept with each test run. Cut-off was calculated and reporting of results were done as positive, negative and equivocal as per the manufacturer's guidelines provided along with the kit.

Dark field microscopy (DFM) was used as a confirmatory test to support the ELISA technique in the diagnosis of leptospirosis.

Dark field microscopy (DFM) is a cost-effective and rapid technique, though it requires about 10 leptospires / ml to be seen by DFM. (29).

Procedure for DFM

All HIV positive patients referred from ART centres and HIV negative patients from OPD were subjected to a questionnaire and those meeting the criteria had each 4ml of blood sample drawn from them and put into EDTA container. The sample was then centrifuged at 1000 rpm for 15 min. After this, 10 µl of Buffy coat or plasma was transferred to a new, clean slide and a cover slip was placed over it and the preparation was examined under dark field microscope. Then the remaining plasma was spun at 3000-4000 rpm for 20 min, at the end of which the supernatant was discarded and wet preparation was done with a drop of sediment which was examined under a dark field microscope. (26) The sample was reported negative if no spirochete were observed after screening of approximately 100 fields in each of the preparations.

Data management and statistics

Raw data and results from patients were edited for consistency and legibility on a daily basis. For qualitative data, the close ended responses were pre-coded before the interview to ensure easy entry and analysis of data using statistical package for social science (SPSS) Computer Software and center for evidence-based Medicine (CEBM) calculator. Serological and microbiological data was entered on the data sheet and was used for analysis. Results obtained from the analyzing machines were tabulated in the data sheets. All the parameters were normally distributed and hence reported as the mean +/- standard deviation. The significance of the differences between patients and controls for

normally distributed parameters were determined using the independent samples T-test for continuous variables and the Chi-square test for categorical variables. Risk factors and patient attributes associated with leptospirosis in HIV patients were determined by logistic regression analysis. Odds ratios and their 95% confidence intervals were reported.

Sensitivity, specificity and positive predictive values for the dark field microscopy as a reliable confirmatory test for leptospirosis were calculated from a 2 by 2 table computed in CEBM statistics calculator. P-Values of less than 5% were taken as significant.

Results

This study consisted of a total number of 282 participants. 150 of them were HIV positive patients and 132 were controls, all aged between 18 and >65 years. The majority of the participants in this study were in the age range of 25-34 years (74 out of 282) as indicated in figure 1. The median age in this study was 44.

This study further reviewed that people living with HIV had higher cases of leptospirosis (50/150) than those who did not have HIV, because, only 15 cases were found to be leptospirosis positive out of the total number of 132 controls enrolled in the study. Figure 2. Graphically demonstrates the association between HIV and the probability of developing leptospirosis with the P Value of 0.003 denoting the statistical significance in the distribution of leptospirosis in the cases and controls.

This study comprised of 150 HIV positive participants, 50 of them were found to have leptospirosis. 35 were females representing 23% while 15 were males representing 10% respectively. According to these results, Leptospirosis cases were found to be more among the female HIV positive participants than males HIV positive participants and the difference was significant (P value 0.002) as indicated in figure 3.

From the total number of 282, fifty (50) cases of leptospirosis from HIV positives represented 18% positivity rate and the 15 cases diagnosed from the non-HIV patients represented 5%. Overall, the mean leptospirosis distribution in HIV patients (18%) was significantly higher (P – value 0.001) than in the control participants (5%) as indicated in figure 4.

Table1: illustrates the mean Leptospirosis profile (ELISA, DFM) in patients with HIV and control subjects by stating the statistical significance of the screening (ELISA) test and the confirmatory (DFM) test in terms of the P – value

Table 2; illustrates cross tabulations of the Elisa test in comparison with the dark field microscopy (DFM). The table further reviews that 65 (23%) of the participants were found reactive with ELISA test and deemed to have leptospirosis.

Out of these results, sixty (21%) participants were true positives, implying that both the ELISA and DFM were positive. 5% of the results were false positive test results with ELISA but the patients did not have leptospirosis because the DFM (confirmatory) was negative.

Results from two (1%) participants were false negative, implying that ELISA test results were negative but participants had leptospirosis in the actual fact. ELISA results from two hundred and fifteen (76%) participants were true negatives because both the ELSA and DFM were negative implying that the patient did not have leptospirosis.

DFM had good sensitivity (97%), specificity (98%) PPV (92%) NPV (99%) and efficiency of (97%) as indicated in table 3 respectively.

Discussion

This study revealed that patients aged 25 years and above were at risk of developing leptospirosis than those who were below 25 years old. These results were consistent with the study done by Brenner DJ et al., (2014) who found that the risk group was above 55 years. However, the reduction in the risk age group of leptospirosis observed in this study may be due to a number of factors including HIV itself, poor hygiene, lack of protective wear when working with potentially infectious materials as a result of poor knowledge on the complications of leptospirosis to some extent. On the other hand, the risk of leptospirosis with increasing age observed by Brenner could be attributed to changes that occur in the immune system as a result of aging tilting the scale to leptospirosis in older patients.

This study reviewed that leptospirosis was related to the HIV status of the patients. Respondents with HIV had a higher positivity rate 50/282 (18 %) than control participants 15/282 (5 %). The difference was significant X^2

= 95.92, P = 0.003. This result correlates with David, A et al, (2015) who found an association of 24%, this shows that there is a significant correlation between HIV and leptospirosis among people infected with HIV in Zambia as compared to the general population. Leptospiral infection in humans causes a range of symptoms, and some infected persons may have no symptoms at all. The disease begins suddenly with fever accompanied by chills, intense headache, severe muscle aches, abdominal pain, and occasionally a skin rash. (8) These symptoms are non-specific to leptospirosis and can occur in other infectious diseases. This clinical feature can mislead a doctor to diagnose the disease as malaria, dengue or yellow fever which have similar symptoms. Severe leptospirosis was also associated with liver, kidney, lungs, and brain damage. For those with signs of meningoencephalitis, altered level of consciousness can occur (9). A variety of neurological complications can occur such as hemiplegia, transverse myelitis, and Guillain-Barre syndrome (10).All these signs and symptoms combined may be mistaken for adverse drug reaction due to ant retro viral treatment (ART) if differential diagnosis is not done to rule out other conditions with similarity and thus raising the number of missed leptospirosis cases (21). This in turn leads to high mortality rate among HIV patients. On increased cases of neurological complications, similar finding was previously reported by Alison *et al.* (2016), where they reported 23% of HIV patients presenting with meningoencephalitis and hemiplegia. However, the Relationship between HIV, leptospirosis and neurological complications has not been investigated (11). So, the present study was done to determine the epidemiology of leptospirosis in HIV patients and have an opportunity to understand the relationship between the two conditions and its possible use as a tool to reduce mortality in HIV patients on ART.

The mean leptospirosis positivity rate in female patients with HIV 35/150 (23%) was significantly higher than in the male patients 15/150 (10%), P – Value 0.002. The differences revealed in the positivity rates of leptospirosis between males and females correlate very well with results obtained by Costa F. et al (2015) among Nigerian HIV adult patients with leptospirosis in which positive cases were high in females than males. The main reason why females with HIV tend to have high cases of leptospirosis than males with the same condition is still unclear. This study reveals that the proportion of participants who had leptospirosis differed significantly among different age groups in HIV positive patients. The proportion of HIV positive patients who had leptospirosis was in the age range of 18 – >65 years. The age group with most cases was 25 – 34 with 74 cases (26%). Results obtained in this study correspond to those obtained by Kimura M et al., (2010) who reported a correlation between old age and leptospirosis. These results were also consistent with the study done by Alison, B et al., and (2015) who found that $\geq$65 years was the most vulnerable age group. Old age tends to decrease resistance against most infections in both males and females due to a number of factors such as atrophying of the thymus were the T lymphocytes are produced which are key in the adaptive immune system. These early hemodynamic changes facilitate the reduction in the production of important immune cells such as the thymocytes. The pick incidence of leptospirosis (42 years) observed in this study, could be associated with the occurrence of early complications which lead to early death as indicated by the decline in the study participants above the age of 55 years.

Leptospirosis is considered as one of the neglected diseases in Africa (25). It is associated with high mortality and morbidity in HIV patients mostly due to misdiagnosis arising from its manifestations which are smiler to other conditions such as malaria. There is currently no diagnostic algorithm and outlined treatment protocol for leptospirosis in Zambia and many other African countries (26).

Current Standard of care for leptospirosis is that Doxycycline is provided once a week as a prophylaxis to minimize infections during outbreaks in endemic regions (27). However, there is no evidence that chemoprophylaxis is effective in containing outbreaks of leptospirosis (9). Pre-exposure prophylaxis may be beneficial for individuals traveling to high-risk areas for a short stay (29).

Effective rat control and avoidance of urine contaminated water sources are essential preventive measures. Human vaccines are available only in a few countries, such as Cuba and China. (30). Animal vaccines only cover a few strains of the bacteria. Dog vaccines are effective for at least one year (27).

For treatment purposes, Effective antibiotics include penicillin G, ampicillin, amoxicillin and doxycycline. In more severe cases cefotaxime or ceftriaxone is preferred.

The motivation for this study was the mounting evidence that preventive measures and treatment for leptospirosis are cheap and readily available. The main challenge in the current care of the disease is mainly misdiagnosis and giving wrong treatment to patients hence contributing to the high mortality observed in this study. These findings are consistent with Haraji et al 2011 results were the main problem in his study was due to lack of approved diagnostic methods in the management of leptospirosis hence most of them were treated for other conditions. The actual cause of high leptospirosis positivity rate in HIV patients is not very clear though Bharti AR et al. suggested that it could partly be due to the disruption in the release of signals by the T helper cells of the adaptive immunity system and this could result in the loss of stimulus to attack invading pathogens by the adaptive immune system. Bharti AR et al (2013) observed that there was a significant high level of positive leptospirosis cases in HIV patients especially in those patients with long term HIV infection and chronic complications. This is consistent with the results of this study. Izurieta R et al., (2014) found that epidemiology of leptospirosis confirmed by DF microscopy was independently associated with other opportunistic complications and suggested that leptospirosis be considered as a separate risk opportunistic infection in HIV patients.

The dark field microscopy had a sensitivity of 97% (95% CI [88.0 – 96.7]), PPV 92% (95% CI [91.9 – 98.7]), NPV 99% (95% CI [83.6 – 94.5]) which were all acceptable parameters to support the reliability and suitability of DFM as a suitable test in determining the epidemiology of leptospirosis.

This study revealed that DFM test had few false negative results leading to high and better sensitivity of 97% (95% CI [88.0 – 96.7]) this implies that the results obtained in this study were reliable and a true reflection of the actual distribution or epidemiology of leptospirosis in the population. DFM test is capable of detecting 97% of leptospirosis cases among HIV patients and only 2% will be missed out as this will be reported as negative. Therefore, DFM test has a high probability of detecting and confirming leptospirosis in HIV patients.

DFM test also had an acceptable specificity of 97% (95% CI [88.8 – 98.2]). This means that, the probability of HIV patients not having leptospirosis was 97% which means 5 (2%) tests gave false positive results.

DFM test had a high PPV 92 % (95%CI [91.9 – 98.7]). This can be interpreted to mean that 60 (92%) positive DFM test results were truly leptospirosis cases.

99% NPV results obtained for DFM in this study means that 215 (99%) of negative DFM test results were true negatives (no leptospirosis) while 2 (1%) were false negatives (had leptospirosis). From the available literature searched so far, no diagnostic study has been done to specifically evaluate the epidemiology of leptospirosis in the population of HIV and non-HIV communities of Kabwe or any other community elsewhere. Long T.W., (2009) reported that the acceptable sensitivity, PPV and NPV should be above 90%. From the results obtained in this study, DFM was found to be a suitable diagnostic and confirmatory test for leptospirosis in HIV patients because all parameters used for detecting suitability were above 90%. Positive and negative predictive values vary according to the prevalence of the condition under study (3). Therefore, it would be wrong for predictive values determined for one population to be applied to another population with a different prevalence. In this case, DFM test results for the determination of the epidemiology of leptospirosis could be used among HIV patients and not the general population because leptospirosis may be absent hence low predictive values even if the test is highly sensitive and specific.

Conclusion

The overall study of 282 participants revealed that HIV patients despite being on ART had higher chances of contracting leptospirosis (18 %) than healthy non-HIV control participants (5 %). Using diagnostic sensitivity and specificity, PPV, NPV and efficiency, it was found that DF microscopy was a reliable test in the confirmation of leptospirosis cases in all the participants. In addition, DF is cheap, readily available as part of the routine microbiology test and easy to perform. Therefore, this study recommends that a lager

study involving bigger population be done and testing algorithm for leptospirosis be put in place in various hospitals in Zambia and Africa as part of the standard care for HIV patients. This will in turn reduce on the mortality levels in HIV patients.

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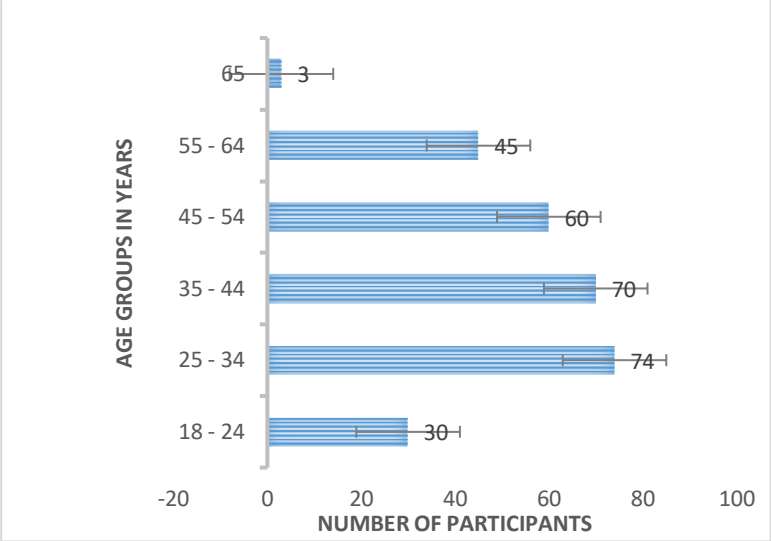

Figure.1. Age distribution (according to years) of respondents

A total number of 150 HIV patients and 132 controls (282 participants), aged between 18 and >65 years participated in this study. The majority (74) of participants were in the age range of 25-34 years as indicated in figure 1 above.

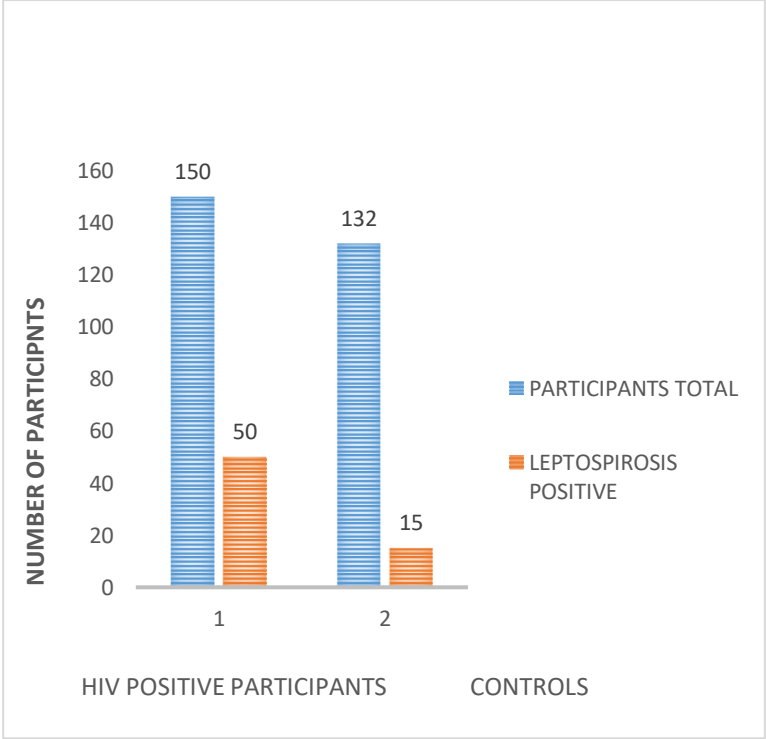

Figure 2. Association between HIV status and leptospirosis

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The figure above indicates that out of the 150 HIV positive patients who participated in the study, 50 were positive for leptospirosis while out of the total of 132 control participants only 15 had leptospirosis. This is an indication that leptospirosis is highly prevalent among HIV patients than the non-HIV patients.

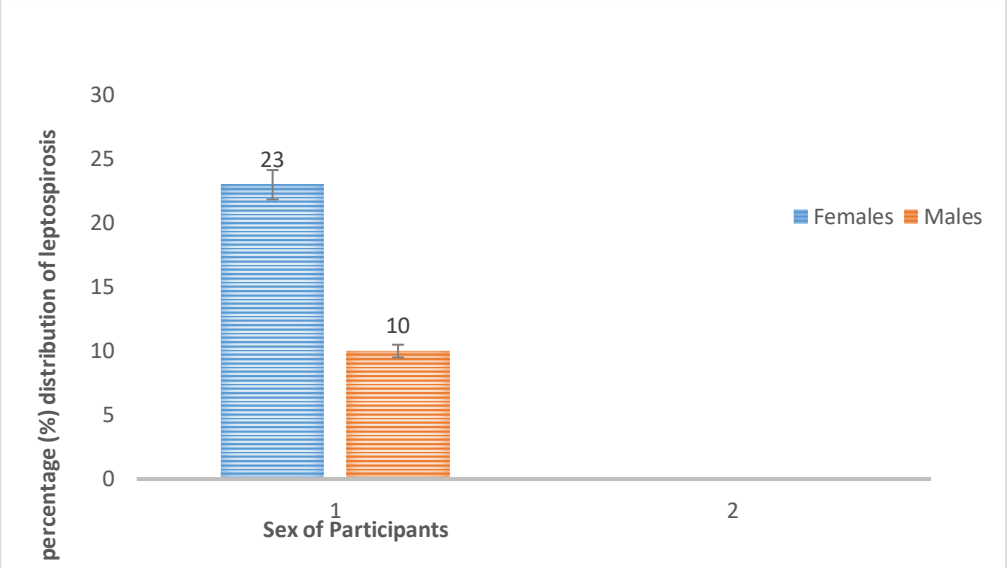

Figure 3. Comparison of percentage distribution of leptospirosis cases in male and female participants

The above figure indicates that the mean distribution of leptospirosis for females with HIV (23%) was significantly higher (P value 0.002) than in the male HIV positive patients (10%).

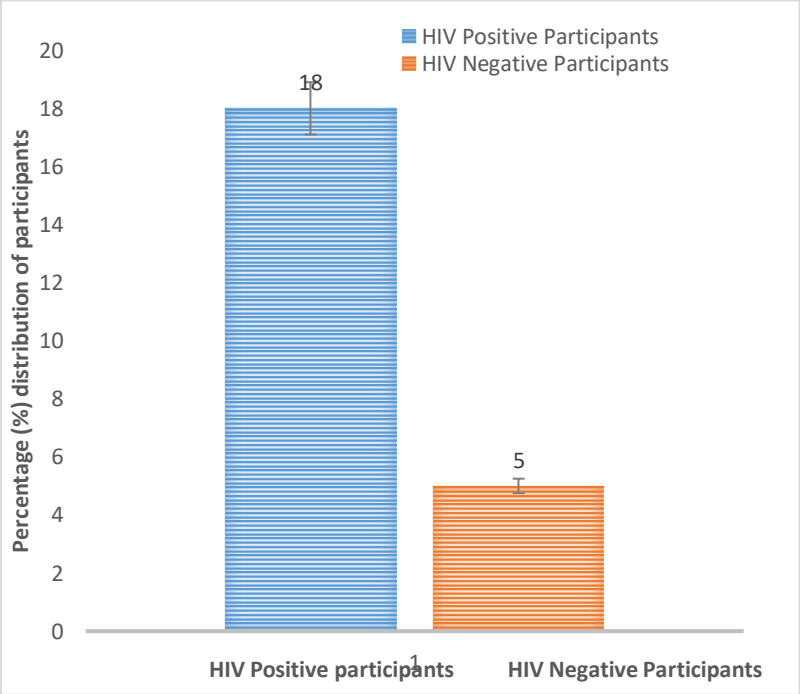

Figure 4. Overall distribution of leptospirosis in HIV positive patient s and control participants.

The overall mean leptospirosis distribution in HIV patients 50/282 (18%) was significantly higher (P – value 0.001) than in the control participants 15/282 (5%) as indicated in figure 4 above.

Table1. Mean Leptospirosis profile (ELISA, DFM) in patients with HIV and control subjects

	Status	**Number of participants**	**Mean (±SD)**	**P - Value**
ELISA*	Control HIV	132 150	250 (±+2.1) 730+4.0	0.003
DFM****	Control HIV	132 150	14.7+3.8 32.2+4.2	0.001

Key: ELISA: Enzyme linked immunosorbent assay

DFM: Dark field microscopy

Table 2. Comparison of ELISA and DFM test results in HIV? Patients with Leptospirosis

ELISA test	DARK FIED MICROSCOPY (DFM)		Total
	Leptospirosis	**No leptospirosis**	
	Leptospires observed	No leptospires observed	
High tests	**TP** 60	**FP** 5	**TP** + **FP** 65
Low tests	**FN** 2	**TN** 215	**FN** + **TN** 217
Total	**TP** + **FN** 62	**FP** + **TN** 220	**TP** + **FP** + **FN** + **TN** 282

Key: TP: True positive = test positive in actually positive cases,

TN: True negative = test negative in actually negative cases,

FP: false positive = test positive in actually negative cases,

FN: false negative = test negative in actually positive cases.

Calculations

(a) Sensitivity = TP/TP + FN x 100 = 60/62 x 100 = 97%

(b) Specificity = TN/FP + TN x 100 = 215/220 x100 = 98%

(c) PPV = TP/TP + FP x 100 = 60/65 x 100 = 92%

(d) NPV = TN/TN + FN x 100 = 215/217 x 100 = 99%

(e) Efficiency = TN + TP/TP + FN +FP + TN x 100 = 275/282 x 100 = 97%