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Prevalence of *Treponema pallidum* among Patient samples tested in the Haematology Laboratory, Usmanu Danfodiyo University Teaching Hospital, Sokoto

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Abstract

Syphilis is a sexually transmitted disease (STD) caused by the bacterium Treponema pallidum. Syphilis can cause long-term complications if not adequately treated. There was paucity of data on prevalence of syphilis infection in Sokoto, north western Nigeria. The aim of this study is to determine the prevalence of syphilis among 400 blood specimens received at the Haematology Laboratory in Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto. Gender distribution of subjects indicated that 240 were male while 160 were females. Syphilis was determined using CTK Biotech, Rapid test kit. Data was analyzed using SPSS ver. 20. There was zero prevalence of syphilis among blood specimens received. In conclusion, this study indicates a significantly low prevalence of syphilis among samples tested in the Haematology Laboratory in Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto. There is need however for laboratory staff to continue to wear relevant personal protective equipments as well as observe universal precaution when dealing with patient samples.

Key words: *Treponema pallidum, Patients, Haematology Laboratory, Usmanu Danfodiyo University Teaching Hospital, Sokoto.*

Introduction:

Syphilis is transmitted from person to person by direct contact with a syphilitic sore, known as a chancre (Peter *et al.*, 2012). Chancres occur mainly on the external genitals, vagina, anus, or in the rectum. Chancres also can occur on the lips and in the mouth. Transmission of syphilis occurs during vaginal, anal, or oral sex. Pregnant women with the disease can transmit it to their unborn child (CDC,

2014). Various studies indicated risk of syphilis transmission via transfusion of untested blood and blood product (Erhabor *et al.*, 2012).

In Lagos, Nigeria; 20% patients comprising 10% males and 10% females were positive for syphilis antibodies (Opara *et al.*, 2013). A cross-sectional study in Nigeria at elementary stages of setting up of the national blood transfusion service to detect antibodies to *Treponema pallidum* using Clinotech syphilis test strips involving a total of 1,410 apparently healthy prospective blood donors yielded an overall sero prevalence of 1.1% (Erhabor, 2012). Studies to investigate the risk of transfusion-transmissible syphilis among blood donors in Ghana have indicated antibody sero prevalence rate of 13.5% for *Treponema pallidum* (Ampofo *et al.*, 2002). Similarly, a study of 252 first-time donors in Cameroon has indicated a 9.1% prevalence rate for syphilis (Mbanya *et al.*, 2003). A previous study in Mali to determine the risk of transfusion-transmissible syphilis infection among Malian blood donors showed a sero prevalence of 0.3% and a higher risk among donations from first time and replacement donors compared to voluntary repeated donors (Diarra *et al.*, 2009). CDC estimates that, annually, 55,400 people in the United States get new syphilis infections. During 2012, there were 49,903 reported new cases of syphilis. Of syphilis cases, 15,667 were of primary and secondary (P and S) syphilis, the earliest and most transmissible stages of syphilis. During the 1990s, syphilis primarily occurred among heterosexual men and women of racial and ethnic minority groups; during the 2000s, however, cases increased among men who have sex with men (MSM). In 2002, rates of P and S syphilis were highest among men 30–39 years-old, but in

2012, were highest among men 20–29 years-old. This epidemiologic shift reflects increasing cases reported among young MSM in recent years. MSM accounted for 75% of all P and S syphilis cases in 2012 (CDC, 2014). Rates of infection with primary and secondary syphilis increased by 15.2 percent between 2006 and 2007, from 3.3 to 3.8 cases per 100,000 persons (Peter *et al.*, 2012).

Additionally, the CDC recently reported a 23 percent increase in congenital syphilis in the United States, from 8.2 cases per 100,000 live births in 2005 to 10.1 cases per 100,000 live births in 2008 (Peter *et al.*, 2012). During 2012, 322 cases of congenital syphilis were reported. There is paucity on the prevalence of syphilis among patients visiting the haematology unit in the North West geopolitical zone of Nigeria. The aim of this study is to determine the prevalence of syphilis among blood specimens received at the Haematology Laboratory in Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto

Materials and Method:

This was a cross-sectional study, carried out for a period of two months between May and June, 2015. Four hundred (400) patients' specimens sent to the Haematology Laboratory in UDUTH, Sokoto were included to determine the prevalence of syphilis infection. The study was carried out in Sokoto State, North-Western Nigeria. The State lies between latitude 13° 3' 49⁰N, longitude 5° 14' 89⁰E and at an altitude of 272 m above sea level and having borders with the Republic of Niger to the North. It also shares boundary with Kebbi State to the West and South and Zamfara to the South and East. The state covers a total land area of about 28,232.27 square kilometers and a population of 3,696,999 (Sokoto Diary, 2008) based on 2006 census (NPC, 2007), with an estimate of 3% growth per annum, the projected population of Sokoto State in 2015 was 4,800,098. Sokoto metropolis is estimated to have a population of 427,760 people (NPC, 2007) and by the virtue of its origin, the state comprises mostly Hausa/Fulani (Abdullahi *et al.*, 2009) and other groups such as Gobirawa, Zabarmawa, Kabawa, Adarawa, (Zama *et al.*, 2013), Arawa, Nupes, Yorubas, Ibos and others (Shehu *et al.*, 2014). Occupation of city inhabitants include trading, commerce, with a reasonable proportion of the population working in private and public sectors (Sokoto Diary, 2008).

The Sokoto township is in dry Sahel surrounded by sandy terrain and isolated hills. Rainfall starts late that is in June and ends early, in September but may

sometimes extend into October. The average annual rainfall is 550 mm with peak in the month August. The highest temperatures of 45°C during the hot season are experienced in the months of March and April. Harmattan, a dry cold and dusty condition is experienced between the months of November and February (Udo and Mamman, 1993). Modern Sokoto city is a major commerce centre in leather crafts and agricultural products (Sokoto Diary, 2008).

This present study was carried out at the Haematology and Blood Transfusion Department of Usmanu Danfodiyo University Teaching Hospital (UDUTH) in Sokoto Nigeria. The hospital is a tertiary health institution located in Sokoto metropolis committed to the provision of quality tertiary healthcare services to the entire North-Western region and neighboring border country - Niger Republic. The study was conducted on patients' specimens received at haematology laboratory in UDUTH, Sokoto State, for an investigation.

Inclusion criteria:

All consenting patients visiting the Haematology Laboratory for laboratory investigation (s), were consecutively recruited.

Exclusion criteria:

All non-consenting patients were excluded from participating in the study.

Sample Size Determination:

The sample size for the study was determined from a standard formula for the calculation of minimum sample size (Ibrahim, 2009; FMOH, 2010).

Sample size n is given by the formula:

$$n = \frac{Z^2 Pq}{d^2}$$

Where n =sample size, q = complementary proportion of p ($1-P$), P =percentage prevalence, Z = Standard normal deviate at 95% confidence level and d = Tolerable margin of error.

Due to lack of a published data on the prevalence of some blood borne pathogens in Sokoto State, 50% was considered.

$$P = 384$$

The calculated sample size was 384. However, the attrition rate of 10 % (approximately 38 specimens) was added to the sample size. Therefore, 422 specimens were included for the study.

Sampling Techniques:

Random Sampling Technique was adopted. Blood specimen was taken, socio-demographic variables accompanying the specimen was recorded.

Informed consent:

Verbal informed consent was obtained from all subjects whose samples were received in the Haematology Laboratory in UDUTH, Sokoto.

Ethical Approval:

Ethical approval was obtained from the Research Ethics Committee of the UDUTH, Sokoto before the commencement of the study.

Blood Processing:

The blood specimens was collected into the plain tubes/ Ethylene Di-amine Di-Acetic Acid tri-potassium (EDTA K₂) bottles. Samples were centrifuged and the sera/ plasma were carefully drawn into sample bottles and then stored frozen at -20°C until the time for analysis.

Analytical Methods:

Screening for Syphilis was carried out using the CTK Biotech (USA) OnSite screening kit, Enzyme Linked Immuno-Sorbent Assay (ELISA) based method. The principle of the test is based on the fact that when an adequate volume of test specimen is dispensed into the sample pad of the strip, the specimen migrates by capillary action across the strip. Anti-*Tp* (*Treponema pallidum*) antibody, if present in the specimen will bind to the *Tp* conjugates. The immunocomplex is then captured on the membrane by the pre-coated *Tp* antigen, forming a burgundy colored T band, indicating a *Tp* antibody positive test result. Absence of the T band suggests a negative result. The test contains an internal control (C band) which should exhibit a burgundy colored band of the immunocomplex of Goat anti-Rabbit IgG/Rabbit IgG-gold conjugate regardless of color development on the T band. Otherwise, the test result is invalid and the specimen must be retested with another device.

Procedure:

1. The strip was dipped into the specimen for 10 seconds.
2. The strip was removed from the specimen, placed on a flat, dry surface.
3. The test result(s) was read after 10 minutes.

Interpretation of Results:

- a. **Negative Result:** If only the C band is developed, the test indicates that no detectable anti-*Tp* antibody is present in the specimen. The result is negative or nonreactive.
- b. **Positive Result:** If both C and T bands are developed, the test indicates for the presence of anti-*Tp* antibody in the specimen. The result is positive or reactive.
- c. **Invalid:** If no C band is developed, the assay is invalid regardless of color development on the T band as indicated below. Repeat the assay with a new device.

Statistical Analysis:

Data was initially collected on an excel spread sheet and subsequently analyzed using SPSS Version 20. Chi Square test was carried out; while for a value below 5.0, Fisher's Exact Test was used. Data was presented in tables as Mean \pm SD. A *p*-value of ≤ 0.05 was considered as significant in all statistical comparisons.

Results:

In this present study, we determine the prevalence of syphilis among 400 blood specimens received at the Haematology Laboratory in Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto. Subjects were aged 1 to 83 years with a mean age of 29.81 ± 1.40 years. Gender distribution of subjects indicated that 240 were male while 160 were females. Figure 1 show the distribution of subjects based on gender. There was zero prevalence of syphilis among blood specimens received. Table 1 show the prevalence of Syphilis among the subjects. Figure 2 show the age distribution of the subjects.

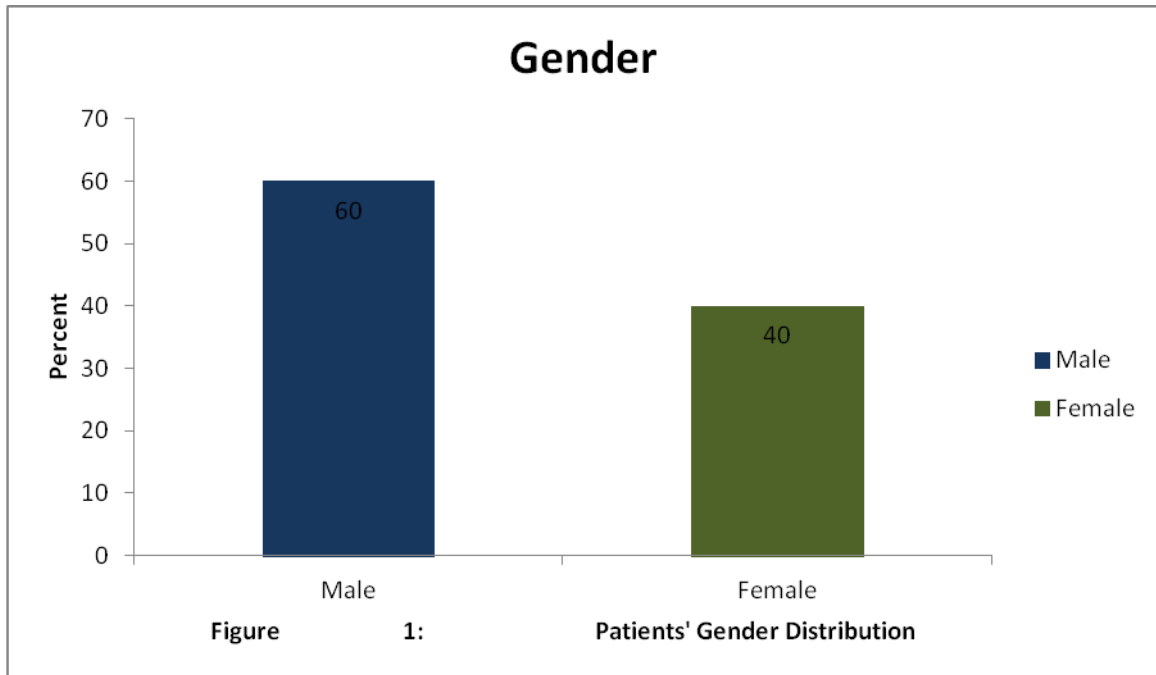


Figure 1: Shows total of four hundred (400) patients’ specimens; consisted of 240 males and 160 females of different age groups were diagnosed for Syphilis 0-17years (Children), 18-24years (Young Adult), 25-39years (Middle Age), 40-64years (Adult) and >65years (Aged).

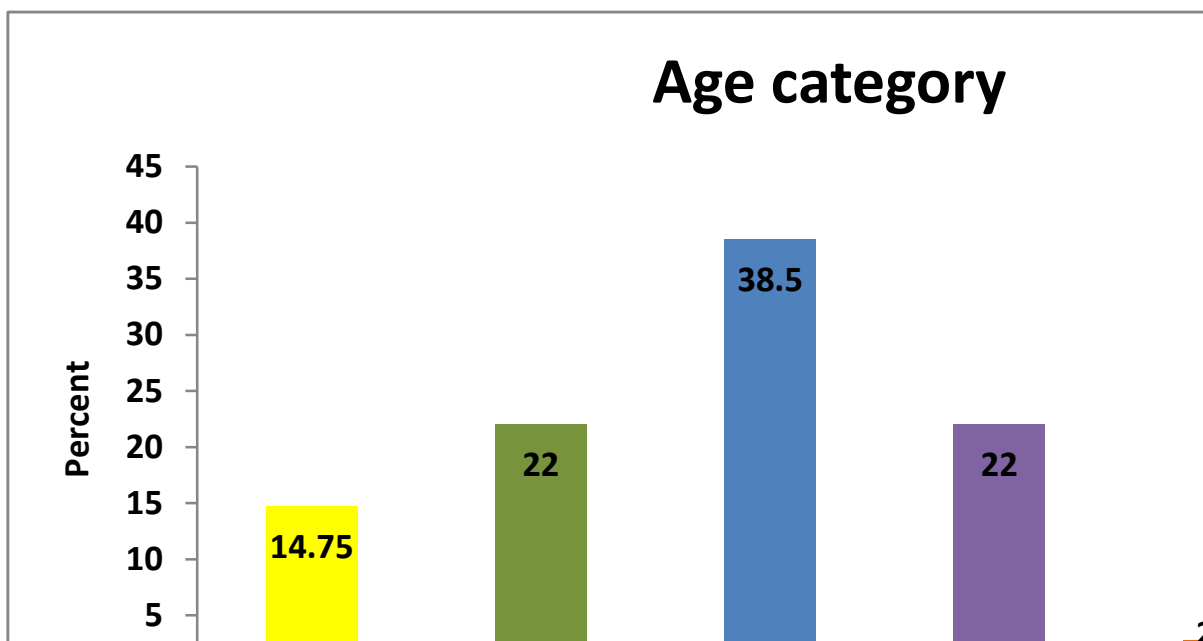


Figure 2: Age distribution of Subjects.

Table 1: Prevalence of Syphilis

Syphilis	Frequency	Percent
Negative	400	100.0
Positive	0	0.0
Total	400	100.0

The above table shows no prevalence of syphilis, since all specimens 400 (100.0%) were negative.

Discussion:

There was zero prevalence of syphilis among the samples tested, since all specimens 400 (100.0%) were negative. Our finding is at variance with previous researches carried out in Lagos, Nigeria in which 20% patients comprising 10% males and 10% females were positive for syphilis antibodies (Opara *et al.*, 2013). A cross-sectional study in Nigeria at elementary stages of setting up of national blood transfusion service to detect antibodies to *Treponema pallidum* using Clinotech syphilis test strips involving a total of 1,410 apparently healthy prospective blood donors yielded an overall sero prevalence of 1.1% (Erhabor *et al.*, 2012). Studies to investigate the risk of transfusion-transmissible syphilis among blood donors in Ghana have indicated antibody sero prevalence rate of 13.5% for *Treponema pallidum* (Ampofo *et al.*, 2002). Similarly, a study of 252 first-time donors in Cameroon indicated a 9.1% prevalence rate for syphilis (Mbanya *et al.*, 2003). A previous study in Mali to determine the risk of transfusion transmissible syphilis infection among Malian blood donors has shown a sero prevalence of 0.3% (Diarra *et al.*, 2009). CDC estimates that, annually, 55,400 people in the United States get new syphilis infections. During 2012, there were 49,903 reported new cases of syphilis (CDC, 2014). The reason for the low prevalence of syphilis in this study is unknown. It may be necessary to carry out a larger study and include the use of a more advanced and sensitive method like ELISA.

Conclusion

Conclusively, this study indicates a low prevalence of syphilis in patient's blood specimens sent or collected in Haematology Department in UDUTH, Sokoto.

Recommendations

It is recommended that laboratory staff to continue to wear relevant personal protective equipments as well as observe universal precaution when dealing with patient samples.

Limitations of the Study

This study screened only syphilis infective organisms. Definitely there were other blood-borne organisms not screened for in this study that may pose occupational risk in the Haematology Laboratory. Secondly, rapid based test was used for testing of the subjects. It may be necessary to carry out a larger study and include the use of a more advanced and sensitive method like ELISA.

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