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STUDY OF THE PREVALENCE OF *TREPONEMA PALLIDUM* CAUSED SYPHILIS IN SUHAG GOVERNORATE THROUGH BLOOD DONOR'S REGIONAL CENTER FOR THE NATIONAL BLOOD TRANSFUSION SERVICES

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ABSTRACT
Treponema pallidum is a motile Gram-negative bacterium causing syphilis. Syphilis is one of the known sexually transmitted infections (STIs) and transfusion transmitted infections (TTIs). Blood samples from 16,000 Egyptian blood donors attending all blood banks in Suhag governorate during the period between January 2015 to December 2015 were screened for markers of infection with hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), and syphilis as per routine check of blood bags for (TTIs). Detection of *Treponema pallidum* was conducted by ELISA technique and 32 cases were found positive. Out of ELISA positive cases; 25 cases were positive when tested with TPHA technique. All cases were found to be negative using PCR as confirmatory test to detect *Treponema pallidum* microbial DNA. Socio demographic variables of detected syphilis cases by ELISA technique were investigated showing that majority of the cases were males, age around twenties, having intermediate level of education and living in rural areas. This study recommends the use of PCR test for syphilis detection either as a sensitive screening routine test or as a confirmatory one. The study emphasizes the importance of a national surveillance system and establishment of reliable databases for development and prevalence of STIs and TTIs. Also, more effective strategies on community education programs and more public health campaigns needed to be conducted to raise awareness about wrong habits and behaviors to bound further spread of these diseases in Egypt.

KEYWORDS: Syphilis, blood banks, TTIs, infection.

INTRODUCTION
Sexually transmitted diseases (STDs), or sexually transmitted infections (STIs), are generally but not exclusively acquired by sexual intercourse. The organisms that cause STIs may pass from person to person via blood, semen, or other body fluids. Sometimes these infections can be transmitted asexually, such as from mother to child during pregnancy or childbirth, or through blood transfusions or shared needles. STIs are not always symptomatic, which is one of the reasons experts prefer the term "sexually transmitted infections" over "sexually transmitted diseases". [1] STIs are causing substantial mortality and morbidity in both adults and newborn and many of them amplify the risk for HIV transmission.[2]

In the developing countries, STIs prevalence is high and increasing by time, making up the second highest cause of healthy life lost in women aged 15 to 45 years, after maternal morbidity and mortality.[3] Moreover, STIs compose an extensive health and economic burden.

In Egypt, the available information on STIs epidemiological situation is limited, unreliable and can't measure the status. STIs epidemiological data are basically driven from fragmented researches focusing mainly on HIV - related aspects neglecting other STIs. The prevalence and incidence of STIs in Egypt have remained generally unknown, and its impact on public health was uncertain despite the obvious communal changes. Egypt faces many challenges maintaining low prevalence of STIs including weak health surveillance system, poor access to accurate health information and care, presence of fear and stigmatization and even criminalization. Egypt was once considered to be an HIV/AIDS low - grade epidemic country, recent data

indicated that Egypt is stepping toward a concentrated HIV epidemic with numerous challenges and barriers for prevention and control in the future.

Causative agents of STIs varies from bacteria (gonorrhea, syphilis, chlamydia), viruses (human papilloma virus, genital herpes, HIV), fungi (candidiasis), parasites (trichomoniasis) and protozoa.[4]

Treponema pallidum is a motile Gram-negative spirochete bacterium which is the causative agent of many diseases such as syphilis, bejel, pinta and yaws.[5] It is generally acquired by close sexual contact. The organism can also be transmitted to a fetus via transplacental route during the later stages of pregnancy, causing congenital syphilis. The helical structure of T. pallidum allows its corkscrew motion through a viscous medium such as mucus. It gains entry to the host's blood and lymph systems through intact mucosa or skin abrasions.[6]

'T. pallidum has one of the smallest bacterial genomes at 1.14 million base pairs, and has limited metabolic capabilities, reflecting its adaptation through genome reduction to the rich environment of mammalian tissue.[7]

Syphilis can be classified into 4 general stages; the primary stage classically characterized by is a small painless sore, called a "chancre". In secondary syphilis a diffuse rash occurs which begins on the trunk but eventually covers the entire body, there may also be sores in the mouth or vagina. In latent or hidden syphilis there are little to no symptoms which can last for years. Signs and symptoms may never return, or the disease may progress to the tertiary (third) stage. In tertiary syphilis; the disease may damage the brain, nerves, eyes, heart, blood vessels, liver, bones and joints. These problems may occur many years after the original, untreated infection.[8]

Transfusion- transmitted infections (TTIs) are infections resulting from the introduction of a pathogen into a person through blood transfusion. A broad range of organisms, including bacteria, viruses and parasites can be transmitted through blood transfusions. The use of a standard donor screening questionnaire besides laboratory tests aid to reduce the risk of TTIs.

The World Health Organization (WHO) recommends that all donated blood is tested for TTIs. These tests include HIV, Hepatitis B, Hepatitis C, Malarya and Treponema pallidum (syphilis).[9] According to the WHO; in 39 countries, blood donations are still not routinely tested for (TTIs) including HIV, hepatitis B, hepatitis C and syphilis; 47% donations in low-income countries are tested in laboratories without quality assurance.[10]

This study is for investigation of the prevalence and spread of syphilis among blood donors in regional center Blood Transfusion Services in Suhag governorate, Egypt.

MATERIALS AND METHODS

Patients

A total number of 16,000 blood donors attending all blood banks in Suhag governorate during the period from January 2015 to December 2015.

Blood was withdrawn from donors aseptically by vein puncture and plasma or serum samples were prepared using standard techniques of preparation of samples for Clinical laboratory analysis.

All blood samples were tested during routine check of blood bags for TTIs including hepatitis B, hepatitis C, HIV and syphilis.

Concerning syphilis; IgG, IgM & IgA antibodies to *Treponema Pallidium* were detected using ELISA technique by qualitative and semi-quantitative Rabid Labs-Syphilis KIT (cat no. DA-SYTA96). Tests were conducted as per kit instructions for users. [11] Briefly; Antibodies against syphilis- if present-in serum samples are captured are captured by recombinant antigens on the plate and marked by HRP recombinant conjugates for visualization with TMB substrate. Kit is provided with positive and negative controls for assay control.

Treponema pallidum hemagglutination antibody (TPHA) test was conducted as well for the qualitative and quantitative determination of antibodies against *Treponema pallidum.* Tests were performed using SYPHILIS TPHA liquid complete kit (cat. No. 50101) according to kit procedure.[12]

Detection of *Treponema pallidum* microbial DNA by (polymerase chain reaction) PCR was performed as confirmatory test for samples found to be positive for *T. pallidum* antibodies upon screening with ELISA and TPHA techniques. Samples were analyzed using PCR Abbot Kit in accordance with kit instructions for use.

RESULTS AND DISCUSSION

Out of 16000 blood samples analyzed for detection of antibodies against Treponema pallidum; 32 cases were found to be positive when analyzed by ELISA technique and upon comparing of different techniques adopted for detection of syphilis; ELISA positive samples were analyzed by TPHA technique. 25 samples were positive by TPHA. All cases were found to be negative using PCR as confirmatory test to detect Treponema pallidum microbial DNA as shown in (table 1).

Table 1: Results of TPHA and PCR among detected syphilis (ELISA positive) cases

Technique	Reactive N (%)	Negative N (%)
TPHA	25(78.1)	7(21.9)
PCR	0(0.0)	32(100)
Total	32(100)	

Generally, a negative antibodies blood test result indicates that it is likely that no infection is present. However, a negative screening test means only that there is no evidence of disease at the time of the test. Antibodies against syphilis may not be detected for several weeks after exposure to the bacteria. On the other hand; a positive result indicates the presence of syphilis antibodies in the blood, but since treponemal antibodies remain positive even after an infection has been treated, it does not indicate whether the person has a current infection or was infected in the past.

PCR test reveals the current presence of the syphilis bacterial DNA, if positive; the person being tested has an infection that requires treatment and if the result is negative this indicates that there is no syphilis infection present.
Socio demographic variables of detected syphilis cases by ELISA technique are showed in (Table 2).

Table 2: showing Socio demographic variables of detected syphilis cases

Variable	No.	Percentage (%)
Gender:		
Male	29	90.6
Female	3	9.4
Age in Years:		
20	23	71.8
25	6	18.8
≥ 30	3	9.4
Educational level:		
Illiterate	2	6.2
Bachelor	10	31.3
Intermediate	16	50
Undergraduate	4	12.5
Residence:		
Urban	13	37.5
Rural	20	62.5
Total	32	100

Data analysis revealed that 90.6% of the positive cases were males, 71.8% were around twenties. Half of the cases were having an intermediate educational level and 62.5% living in rural areas.

Socio demographic analysis is required as it indicates that certain factors may be associated with syphilis infection including older age, education level, geographic location, lower tier of typology.[14]

Blood born infections detected among syphilis cases (ELISA positive) are shown in (table 3).

Table 3: blood born infections detected among syphilis cases (ELISA positive)

Disease	Negative N (%)	Positive N (%)
HBsAg	30(93.8)	2(6.2)
HCVAb	32(100)	0(0.0)
HIVAb1+2	31(96.9)	1(3.1)
Total	32 (100)	

Syphilis infected persons are 2-5 times more eligible for HIV infection. In study of Safety of Blood Transfusion of 308,762 donors conducted in Egypt during the period 2007- 2011 has noted an increasing trend for HIV and syphilis. This elevated burden of HIV between blood donors who represent the healthy sector of the community) emphasizes a clear need start paying attention to the emerging threat of HIV. It is also crucial not ignore the high prevalence of HIV in nearby African countries.[15] Another study showed that Syphilis prevalence in 1990s predicated approximately 53% of the variation in peak HIV prevalence. Also, population with generalized HIV epidemics had a higher prevalence of syphilis in the pre-HIV period.[16]

Another study evaluated factors that influence the likelihood of syphilis infection indicated a significant association between HIV, HBV and syphilis. An elevated rate of syphilis-HBV co-infection was observed in HIV-negative donors and further research revealed a relation between syphilis and specific HBV serological reactivity.[17]

CONCLUSIONS

The study was conducted among donors from blood banks covering Suhag governorate via regional center for the National Blood Transfusion Services to study the prevalence of *Treponema Pallidum.* Different techniques were adopted to detect *T. pallidum* in donor's blood samples giving different results. Hence PCR technique was the most accurate test to give a definitive indication for the presence of current positive case making it a recommended test for syphilis detection either as a sensitive screening routine test or as a confirmatory one. The main limitation of this study is that it was conducted over one year. Only a national epidemiologic study can provide precise information about the overall prevalence of the studied infectious diseases in Egypt but our study can provide a useful indication and emphasizes the importance of a national surveillance system and establishment of reliable databases for development and prevalence of STIs and TTIs. Also, more effective strategies on community education programs and more public health campaigns needed to be conducted to raise awareness about wrong habits and behaviors to bound further spread of these diseases in Egypt.

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