

## Original Article

# Seroepidemiology and Risk Factors Analysis of Syphilis: A Cross-Sectional Study from Peshawar, Pakistan

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## Abstract

**Objective:** To assess the prevalence and determine the risk factors analysis of syphilis in blood donors.

**Methodology:** It was a cross-sectional, prospective, single centre study of blood donors from June 2020 to June 2021. After selection and obtaining informed consent, the required blood samples for screening were collected from the blood donors and tested by Chemiluminescence Microparticle Immunoassay (CMIA) for the detection of antibodies specific to *Treponema pallidum*. All syphilis reactive blood donors filled out a questionnaire used for risk factors analysis.

**Results:** A total of 32,812 blood samples were tested by the CMIA technique for the detection of antibodies against *T. pallidum* of which 272 (0.83%) were reactive while 32,540(99.17%) were non-reactive. Male participants in the study were 32,661(99.54%) while 151(0.46%) were females. Voluntary blood donors comprised of 1,885 (5.74%) while 30,927(94.26%) were replacement donors. In the replacement blood donors category, 262(0.86%) were reactive to syphilis antibodies whereas 10(0.53%) were reactive for syphilis in the voluntary blood donors category. All of the reactive cases were in the male blood donors and no reactive case was detected in female blood donors. Of the 272 reactive blood donors, the majority (39.71%) were from urban and 164 (60.29%) were from rural areas. The majority of the reactive blood donors were between the age of 18 to 37 years.

**Conclusion:** The current study provided a baseline for the rate of local prevalence and risk factors analysis for syphilis. In Khyber Pakhtunkhwa province, syphilis has become prevalent posing a threat to blood safety. Further studies with a higher sample size are needed. A campaign for health education at the national and provincial levels is imperative.

**Keywords:** *Treponema pallidum*, Syphilis, Khyber Pakhtunkhwa, Prevalence, Blood Donors.

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## Introduction

Syphilis remains a leading cause of morbidity and mortality globally. While syphilis infection is easily detectable and treatable, rates of infection remain higher among certain populations in high HDI (human development index) countries, while they remain endemic in low- and middle-HDI countries.

Transfusion of blood is an important intervention for

saving lives, and globally, millions of precious lives are saved each year by using this procedure.<sup>1</sup> A major risk for the global spread of transfusion-transmitted infections (TTIs) is transfusion of unsafe blood and blood components.<sup>2</sup> Among the diseases which can be transmitted through blood, syphilis is very significant, particularly in pregnant women who are generally transfused at the time of delivery due to anaemia.

Authorship Contribution: <sup>1,2</sup>Substantial contributions to the conception or design of the work, acquisition, analysis, or interpretation of data for the work, <sup>2,3</sup>Drafting the work or revising it critically for important intellectual content, <sup>4</sup>Active participation in methodology, <sup>3</sup>Final approval of the version to be published.

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Syphilis is a disease caused by a spirochete, *Treponema pallidum* that was identified by Schaudinn and Hoffmann in 1905.<sup>3,4</sup> It is transmitted via sexual contact and from a mother to foetus vertically during pregnancy or new-born during childbirth. According to the WHO, approximately 6.3 million new cases of syphilis are diagnosed each year, with 90% of these cases occurring in countries with very low HDI (human development index) levels.<sup>5</sup> Three hundred thousand foetal and neonatal deaths are caused by syphilis. Besides, in infants about 215,000 are placed at high risk of death at an early age.<sup>6</sup>

In the United States, the reported cases of Syphilis in 2019 were 129,813.<sup>7</sup> During 2010-2015 in Canada, an increase in the rate of Syphilis from 5.0 to 9.3/100,000 population was recorded.<sup>8</sup> A country-wide retrospective descriptive study of syphilis prevalence was conducted by the Ministry of National Health Services, Pakistan from January to December 2018 in which 0.72% of blood donors were reactive for syphilis.<sup>9</sup> The prevalence of syphilis in blood donors reported by some other studies conducted in different areas of Pakistan were 3.1%, 101.55%, 112.1%,<sup>12</sup> and 0.91%.<sup>13</sup> In blood donors, the overall prevalence of syphilis in Peshawar from June 2016 to May 2020 was reported at 0.91%.<sup>14</sup>

Syphilis is a common STD (sexually transmitted disease) worldwide, and its prevalence has increased in Pakistan in recent years.<sup>15-17</sup> In a population the burden of this disease can be estimated by the screening of healthy blood donors that in addition also ensures the safety of blood.<sup>18,19</sup> In blood donors, it was revealed that most reported cases have occurred during the primary and secondary stages of Syphilis.<sup>3,20</sup>

Under the blood safety legislation of Pakistan, screening of syphilis is mandatory. The national strategies to decrease the chances of Syphilis transmission through blood and its components are pre-donation screening of blood donors by using questionnaires to identify those who are at more risk of TTIs and screening of the blood for TTIs by using laboratory techniques that are more specific and sensitive.<sup>21</sup> The important risk factors for syphilis are marital status, sex, age, educational level, occupation, nationality and other important behaviours including sexual contact with syphilis patients, paid sex, condom use, body fluid contact, dental treatment, injection,

acupuncture, transfusion, dental treatment, tattooing, sharing razor and ear piercing.<sup>22</sup>

It is vital to estimate the syphilis burden, monitor trends of epidemics, and evaluate control strategies. For better control of syphilis, identification and analysis of risk factors are very important. Therefore, the current study was performed to assess the prevalence and risk factors analysis of syphilis in the blood donors of Khyber Pakhtunkhwa.

## Methodology

The present study was a prospective cross-sectional study performed at the Regional Blood Centre (RBC) in Peshawar, from June 2020 to June 2021. Blood donors' selection was done according to the national guidelines of the Safe Blood Transfusion Programme of Pakistan (2020). From all blood donors, informed consent was taken. Before the blood donation, every blood donor was asked to fill out a detailed questionnaire about their health history.

Blood donors who fulfilled the blood donor selection criterion of the national guidelines were included. According to these guidelines, if they were 18-60 years old, physically fit, weigh >50 kg, HB >12.5 g/dl, and not malnourished. All patients with no history of asthma, jaundice, malaria, not anaemic, having no engagement in high-risk behaviour (i.e. drug abuse and unsafe sex) and having no history of HBV, HIV, syphilis, and HCV were included. Blood donors who did not fulfil the selection criterion were excluded.

CMIA was used for screening of blood donors for syphilis and a total of 32,812 samples were tested by this technique. By using ARCHITECT syphilis *T. pallidum* (TP) assay, in human serum or plasma, the qualitative detection of antibodies against TP was carried out.<sup>23</sup>

All syphilis reactive blood donors were called by the Health Education Officer (HEO)/Genetic Counsellor of the Peshawar Regional Blood Centre for counselling. A questionnaire specially designed for risk factors was filled out according to the information shared by the reactive blood donors with the Health Education Officer (HEO)/Genetic Counsellor during their counselling about the disease. The reactive blood donors were counselled by the concerned specialist physician on disease prevention, further confirmatory tests, and treatment.

## Results

A total of 32,812 blood donors were screened by CMIA for anti-*T. pallidum* antibodies of which 272 (0.83%) were reactive while 32,540(99.17%) were non-reactive. Of these 32,812 blood donors screened, 32,661 (99.54%) were males and 151 (0.46%) were females. Voluntary blood donors were 1,885 (5.74%) while 30,927 (94.26%) were family/replacement donors. When these reactive blood donors were further analysed, it was concluded that in male blood donors, 272 (0.83%) cases while in female blood donors, zero cases were reactive for syphilis. On further analysis, it was concluded that the syphilis reactive cases in both voluntary and replacement blood donors were 10 (0.53%) and 262 (0.86%) respectively (Table I & II).

When these 272 reactive blood donors were analysed for their district-wise distribution, their distribution in different districts of Khyber Pakhtunkhwa varied as shown in Table III.

When these 272 reactive blood donors were analysed based on age groups, in the age group 18-27 years were 82 (30.14%), 118 (43.38%) in the age group of 28-37 years, 60 (22.06%) were in the age group of 38-47 years, and 12 (4.41%) were in the age group of 48-57 years. In the age group 28-37 years, more reactive cases 118 (43.38%) were recorded while in the age group 48-57 years, the least number of syphilis reactive cases 12 (4.41%) were recorded. Among reactive blood donors, 64 (23.53%) were single, 208 (76.47%) were married and there was no reactive case in the divorced/separated category of blood donors. Married blood donors were more affected as compared to single blood donors ( $P=0.001$ ).

When the reactive blood donors were assessed based on sexual history, syphilis reactive cases were more in blood donors who had sex as compared to blood donors who never had sex. Syphilis reactive cases were high in blood donors having unprotected sex. When these reactive blood donors were analyzed

**Table I: Total number of blood donors tested for syphilis by CMIA (n=32,812)**

Blood donors screened	Syphilis Reactive	Syphilis Reactive	Non-Males	Females	Voluntary blood donors	Replacement blood donors
32,812	272(0.83%)	32,540(99.17%)	32,661(99.54%)	151(0.46%)	1,885(5.74%)	30,927(94.26%)

**Table II: Analysis of reactive blood donors**

Total male donors	Reactive male donors	Total female donors	Reactive female donors	Voluntary blood donors	Reactive voluntary blood donors	Total replacement blood donors	Reactive replacement blood donors
32,661	272 (0.83%)	151	0.0%	1,885	10 (0.53%)	30,927	262 (0.86%)

**Table III: District wise distribution of *T. pallidum* reactive blood donors in Khyber Pakhtunkhwa (n=272)**

District	Number Reactive Donors	Percentage
Nowshera	3	1.10
Charsada	9	3.30
DI Khan	3	1.10
Mardan	12	4.41
Chitral	3	1.10
South Waziristan	6	2.20
Bannu	9	3.30
Dir Upper	3	1.10
Dir Lower	9	3.30
Bajawar	4	1.5
Batagram	3	1.10
Khyber	6	2.20
Shangla	3	1.10
Swabi	6	2.20
Lakimarwat	3	1.10
Swat	9	3.30
Buner	3	1.10
Malakand	3	1.10
Kohat	15	5.51
Peshawar	160	58.82

based on drug history, only 18(6.61%) were illicit drug users and there was no intravenous drug user (IDU) (Table IV).

The analysis of reactive cases with the occupation, risks/history of infections, education levels is shown in Table IV.

## Discussion

A blood transfusion that is not safe results in a heavy cost both from an economic and a human point of view, not only for the patients, but also for their relatives and their communities. The safety of blood transfusion has reached at a very elevated level, yet, some residual risks of transfusion-transmitted infections remain in discussion before blood donation. The vulnerable populations of syphilis is the same as HIV/AIDS, with analogous biological and behavioural factors.<sup>18</sup>

**Table IV: Risk factors/demographic characteristics associated with syphilis infection in blood donors of Khyber Pakhtunkhwa (n=272).**

Risk Factors/Demographic Characteristics		Number	Percentage
Age group (years)	18-27	82	30.14
	28-37	118	43.38
	38-47	60	22.06
	48-57	12	4.41
Sex	Male	272	100
	Female	0.0	0.00
Materials	Single	64	23.53
	Married	208	76.47
	Divorced/Separated	0.0	0.00
Sexual history	Ever had sex	208	76.47
	Never had sex	64	23.53
	Multiple sexual partners	39	14.33
	Prostitution	0.0	0.00
	Unprotected sex	184	67.64
	Men who have sex with men	12	4.41
Drug history	Illicit drug use	18	6.61
	Intravenous drug use	0.0	0.00
Risks/history of infections	Residence in highly prevalent areas	160	58.82
	HIV infection	0.0	0.00
	Presence of other STIs	0.0	0.00
	Previous history of STIs	0.0	0.00
	Health care professionals who are predisposed to occupational risk	0.0	0.00
	Low socioeconomic status	254	93.38
	Skin scarification (tattooing, blood rituals)	0.0	0.00
	History of blood transfusion	24	8.82
Occupation	Jobless	18	6.61
	Shopkeeper	45	16.54
	Student	24	8.82
	Driver	73	26.83
	Labour	55	20.22
	Farmer	12	4.41
	Public sector employee	24	8.82
	Private s sector employee	12	4.41
	Contractor	6	2.20
	Property dealer	3	1.10
Educational level	No Formal education	47	17.27
	Primary school	44	16.17
	Middle school	54	19.85
	Secondary school	73	26.83
	Higher secondary school	39	14.33
	University (graduate)	15	5.51
Residence	Rural	164	60.29
	Urban	108	39.71

In modern medicine, blood is a life-saving procedure. The safe supply of blood and blood components is ensured by the proper screening of blood. The

prevalence of TTIs in a healthy population is an indicator of proper screening of blood donors. In Pakistan, the majority of the blood donors are first-timers and the presence of an infection in a community can be estimated from their screening for TTIs.<sup>18</sup>

The reactive cases of syphilis (*T.pallidum*) in the present study were 272(0.83%). The findings in our study were higher when it was compared with results of different studies conducted in some developed countries, including the USA(0.16%),<sup>25</sup> Israel(0.047%),<sup>26</sup> and Saudi Arabia (0.044%).<sup>27</sup> When our results were compared with studies from different countries of the African continent, the prevalence of syphilis in blood donors was very high than the results of the present study, for example in Burkina Faso(1.5%),<sup>28</sup> Nigeria(3.1%)<sup>29</sup> and Angola(20.0%).<sup>30</sup> Our results were a little high when compared with a study conducted by the Safe Blood Transfusion Programme of Pakistan in which the prevalence of Syphilis in Pakistani blood donors was 0.72%.<sup>9</sup> When we compared our study with other earlier studies from different cities of Pakistan, the prevalence rates were Karachi(0.91%),<sup>13</sup> Karachi (2.1%),<sup>12</sup> Islamabad 1.55%,<sup>11</sup> Peshawar (0.43%),<sup>31</sup> Lahore (0.5%),<sup>32</sup> Lahore (2.25%).<sup>33</sup> Our result was high (0.83%) as compared to an earlier study from Peshawar where it was 0.43% and this difference may be due to the difference of time between these two studies, our study was conducted in 2020-2021 while the other study was conducted in 2008 to 2011.

In the present study, most of the syphilis reactive donors were between the age of 18-27 and 28-37 years, all of the syphilis reactive cases were reported in the male, most of the reactive cases were reported in married blood donors, drivers, labours, blood donors had education less than SSC and blood donors from urban areas. In the United States, a study was conducted in which the high prevalence of syphilis cases was reported in blood donors who were male, having aged > 50, blood donors who donated for the first time, had a low level of education, was born outside the US, and had positive serological tests for HCV and HIV.<sup>24</sup> In Canada, the highest number of Syphilis cases were reported in males, 25–29 and 30–39 years aged persons.<sup>8</sup> A study was conducted in Brazil in which the blood donors at high risk of syphilis were male, had age <40 years, 11 years of education, married, had multiple sexual partners, MSM, not using condoms, had a history of STI and ever used illicit drugs.<sup>35</sup> With the sexual realization becoming more and

more exposed, most individuals use mobile phones and software to search for sexual partners, which upsurges the people of multi-sex partners. Multiple sexual partners are a substantial risk factor for syphilis infection.

A study was conducted in Ethiopia in which the prevalence of syphilis was significantly increased in blood donors who were construction workers and labourers as compared to students from different educational institutions.<sup>36</sup> A study was conducted in Burkina Faso in which the highest prevalence of syphilis was detected in blood donors who were male, aged 20-29 years, from the urban area and were first-time blood donors.<sup>37</sup> In Kenya, a study was conducted in which the history of blood transfusion in the past and being married were the high-risk factors associated with positive syphilis cases.<sup>38</sup>

A study was conducted in Istanbul, Turkey in which the persons at high risk of syphilis were male, having aged 20 to 40 years, had education up to primary school, married, homosexual, had 2-10 sexual partners and those who were not using condoms.<sup>39</sup> A study was conducted in Isfahan, Iran on female prisoners in which the high prevalence of syphilis was noted in those who had education up to primary school, were married, had first-time marriage, and had ear piercings.<sup>40</sup> A study was conducted in China in which the blood donors at high risk of syphilis were male, merchant and commercial, having middle school education, reuse of razor, piercing of the ear, tattooing, dental surgery, acupuncture, transfusion, contact with the different the fluid of the body, sex by money, having sexual partners >2, no use of a condom and sexual contact with syphilis.<sup>22</sup> The risk factors findings in the present study were almost similar to a study conducted in Karachi<sup>12</sup> and Faisalabad, Pakistan.<sup>41</sup> In the present study, one of the risk groups for syphilis was drivers which were similar to a study conducted in Rawalpindi/Islamabad in which one of the risk group for syphilis was truck drivers.<sup>42</sup>

Among the blood donors within the different regions of Pakistan and also in other countries in the world, there are differences in the prevalence of syphilis infection and also the risk factors associated with it. This difference may be due to the differences in sexual behaviour, blood donors range, geographical locations of the populations, cultural practices such as practices of marriage, age, the period of the studies and the size of samples.

## Conclusion

In Khyber Pakhtunkhwa province, the risk of transmission of *T. pallidum* through blood transfusion is very high. Regardless of the temperature which is maintained in the blood banks, based on the present study we strongly recommend that all blood banks must screen blood donors before the transfusion of blood, which may curtail the transmission of syphilis by blood transfusion and may have long-term effects. To decrease the risk of this, an increase in voluntary blood donors and female blood donors is strongly recommended. Improvement in monitoring, surveillance and evaluation of transfusion-transmitted infections and sexually transmitted infections is very important. Improvement in reporting and standardization in the testing of syphilis and more focus on strategies that are effective for syphilis control. All of the blood components which are collected from a donor who is reactive to *T. pallidum* must be discarded. The current study provided a baseline for the rate of local prevalence and risk factors analysis for Syphilis. Awareness and sensitization campaign for health education at the national level is the need of time.

## References

1. Manzoor I, Hashmi N, Daud SE, Ajmal SA, Fatima H, Rasheed ZA, Syed SA. Seroprevalence of transfusion transmissible infections (TTIs) in blood donors. *Biomedica*. 2009 Jul;25(10):154-8.
2. Zaid M, Ali M, Afzal MS. HIV outbreaks in Pakistan. *The Lancet HIV*. 2019 1;6(7):e418-9.
3. Gardella C, Marfin AA, Kahn RH, Swint E, Markowitz LE. Persons with early syphilis identified through blood or plasma donation screening in the United States. *The Journal of infectious diseases*. 2002 Feb 15;185(4):545-9.
4. Waugh M. The centenary of *Treponema pallidum*: on the discovery of *Spirochaeta pallida*. *Int J STD AIDS*. 2005;16(9):594-5.
5. Taylor MM, Wi TE. Transforming and integrating STI surveillance to enhance global advocacy and investment in STI control. *Journal of the International AIDS Society*. 2019 Aug;22(Suppl 6).
6. World Health Organization. Global health sector strategy on sexually transmitted infections 2016-2021: toward ending STIs. World Health Organization; 2016.
7. Kreisel KM, Spicknall IH, Gargano JW, Lewis FM, Lewis RM, Markowitz LE, Roberts H, et al. Sexually transmitted infections among US women and men: Prevalence and incidence estimates, 2018. *Sexually Transmitted Diseases*. 2021 Apr 1;48(4):208-14.
8. Choudhri Y, Miller J, Sandhu J, Leon A, Aho J. Sexually transmitted infections: Infectious and congenital syphilis in Canada, 2010-2015. *Canada Communicable Disease Report*. 2018 Feb 1;44(2):43.
9. Waheed U, e Saba N, Wazeer A, Arshad M, Zaheer HA. Epidemiology of syphilis in blood donors in Pakistan. *Global Journal of Transfusion Medicine*. 2020 Jan 1;5(1):100.
10. Nazir S, Pracha HS, Khan A, Nazar A, Fayyaz A, Khan MS, Ahmed S, et al. Prevalence of syphilis in Pakistani blood donors. *Advancements in life sciences*. 2013 Nov 25;1(1):27-30.

11. Saeed M, Hussain S, Rasheed F, Ahmad M, Arif M, Rahmani MT. Silent killers: Transfusion transmissible infections-TTI, among asymptomatic population of Pakistan. *J Pak Med Assoc.* 2017 Mar 1;67(3):369-74.
12. Arshad A, Borhany M, Anwar N, Naseer I, Ansari R, Boota S, Fatima N, Zaidi M, Shamsi T. Prevalence of transfusion transmissible infections in blood donors of Pakistan. *BMC hematology.* 2016 Dec;16(1):1-6.
13. Sultan S, Murad S, Irfan SM, Biag MA. Trends of venereal infections among healthy blood donors at Karachi. *Archives of Iranian medicine.* 2016 Mar 1;19(3):0-.
14. Saba N, Nasir JA, Waheed U, Aslam S, Mohammad I, Wazeer A, Ahmed S, et al. Seroprevalence of Transfusion-Transmitted Infections among Voluntary and Replacement Blood Donors at the Peshawar Regional Blood Centre, Khyber Pakhtunkhwa, Pakistan. *Journal of Laboratory Physicians.* 2021 May 26.
15. Allain JP, Stramer SL, Carneiro-Proietti AB, Martins ML, Da Silva SL, Ribeiro M, Proietti FA, Reesink HW. Transfusion-transmitted infectious diseases. *Biologicals.* 2009 Apr 1;37(2):71-7.
16. Waheed U, Khan H, Satti HS, Ansari MA, Malik MA, Zaheer HA. Prevalence of transfusion transmitted infections among blood donors of a teaching hospital in Islamabad. *Ann Pak Inst Med Sci.* 2012;8(4):236-9.
17. Mahmood H, Ansari US, Aslam M. Prevalence of syphilis and HIV infection in blood donors in cosmopolitan Lahore during the year 2014. *Journal of University Medical & Dental College.* 2015 Dec 3;6(4):42-5.
18. Khan ZT, Asim S, Tariz Z, Ehsan IA, Malik RA, Ashfaq B, Hayat A. Prevalence of Transfusion transmitted infections in healthy blood donors in Rawalpindi District, Pakistan—a five year study. *Int J Pathol.* 2007;5(1):21-5.
19. Lynn WA, Lightman S. Syphilis and HIV: a dangerous combination. *Lancet Infect Dis.* 2004;4(7):456–66.
20. Singh AE, Romanowski B. Syphilis: review with emphasis on clinical, epidemiologic, and some biologic features. *Clinical microbiology reviews.* 1999 Apr 1;12(2):187-209.
21. Waheed U, Ahmed S, e Saba N, Wazeer A. Haemovigilance as a quality indicator in transfusion medicine: Pakistan's perspective. *Annals of PIMS-Shaheed Zulfiqar Ali Bhutto Medical University.* 2020 Apr 28;16(1):46-51.
22. Liu S, Luo L, Xi G, Wan L, Zhong L, Chen X, Gong T, et al. Seroprevalence and risk factors on Syphilis among blood donors in Chengdu, China, from 2005 to 2017. *BMC infectious diseases.* 2019 Dec;19(1):1-8.
23. Attie A, de Almeida-Neto C, S Witkin S, Derriga J, Nishiya AS, Ferreira JE, Costa ND, et al. Detection and analysis of blood donors seropositive for syphilis. *Transfusion Medicine.* 2021 Apr;31(2):121-8.
24. Kane MA, Bloch EM, Bruhn R, Kaidarova Z, Murphy EL. Demographic determinants of syphilis seroprevalence among US blood donors, 2011–2012. *BMC infectious diseases.* 2015 ;15(1):1-9.
25. Drago F, Cogorno L, Ciccarese G, Strada P, Tognoni M, Rebora A, Parodi A. Prevalence of syphilis among voluntary blood donors in Liguria region (Italy) from 2009 to 2013. *International Journal of Infectious Diseases.* 2014 Nov 1;28:45-6.
26. Vera L, Milka D, Nurith SL, Eilat S. Prevalence and incidence of syphilis among volunteer blood donors in Israel. *Journal of blood transfusion.* 2014;2014.
27. Elyamany G. Prevalence of syphilis among blood and stem cell donors in Saudi Arabia: An institutional experience. *Electronic physician.* 2016;8(8):2747.
28. Bisseye C, Sanou M, Nagalo BM, Kiba A, Compaoré TR, Tao I, Simpore J. Epidemiology of syphilis in regional blood transfusion centres in Burkina Faso, West Africa. *Pan African Medical Journal.* 2014;16(1).
29. Okoroiwu HU, Okafor IM, Asemota EA, Okpokam DC. Seroprevalence of transfusion-transmissible infections (HBV, HCV, syphilis and HIV) among prospective blood donors in a tertiary health care facility in Calabar, Nigeria; an eleven years evaluation. *BMC public health.* 2018 Dec;18(1):1-8.
30. Quintas E, Cogle AC, Dias CC, Sebastiao A, da Costa Pereira A, Sarmiento A. Prevalence of Syphilis in Blood Donors in Angola from 2011 to 2016. *Clin Med Rep.* 2018 Jan 1;2:1-4.
31. Attaulah S, Khan S, Khan J. Trend of transfusion transmitted infections frequency in blood donors: provide a road map for its prevention and control. *Journal of translational medicine.* 2012 Dec;10(1):1-5.
32. Manzoor I, Hashmi N, Daud SE, Ajmal SA, Fatima H, Rasheed ZA, Syed SA. Seroprevalence of transfusion transmissible infections (TTIS) in blood donors. *Biomedica.* 2009 Jul;25(10):154-8.
33. Zahoor S, Hameed S, Iqbal W, ur Rehman H, ur Rehman N, Jaral J. Seroprevalence of Syphilis in healthy blood donors of Lahore during year 2016 and 2017; An upcoming problem for Pakistan. *The Professional Medical Journal.* 2020 Jan 10;27(01):138-42.
34. Zaheer HA, Saeed U, Waheed Y, Karimi S, Waheed U. Prevalence and trends of hepatitis B, hepatitis C and human immunodeficiency viruses among blood donors in Islamabad, Pakistan 2005-2013. *J Blood Disorders Transf.* 2014;5(217):2.
35. Ferreira SC, de Almeida-Neto C, Nishiya AS, Oliveira CD, Ferreira JE, Alencar CS, et al. Demographic, risk factors and motivations among blood donors with reactive serologic tests for syphilis in São Paulo, Brazil. *Transfusion Medicine.* 2014 Jun;24(3):169-75.
36. Tessema B, Yismaw G, Kassu A, Amsalu A, Mulu A, Emmrich F, Sack U. Seroprevalence of HIV, HBV, HCV and syphilis infections among blood donors at Gondar University Teaching Hospital, Northwest Ethiopia: declining trends over a period of five years. *BMC Infectious diseases.* 2010 Dec;10(1):1-7.
37. Nagalo MB, Sanou M, Bisseye C, Kaboré MI, Nebie YK, Kienou K, et al. Seroprevalence of human immunodeficiency virus, hepatitis B and C viruses and syphilis among blood donors in Koudougou (Burkina Faso) in 2009. *Blood transfusion.* 2011 Oct;9(4):419.
38. Bartonjo G, Oundo J. Prevalence and associated risk factors of transfusion transmissible infections among blood donors at Regional Blood Transfusion Center Nakuru and Tenwek Mission Hospital, Kenya. *The Pan African Medical Journal.* 2019;34.
39. Aydın ÖA, Karaosmanoğlu HK, Sayan M, İnce ER, Nazlıcan Ö. Seroprevalence and risk factors of syphilis among HIV/AIDS patients in Istanbul, Turkey. *Central European journal of public health.* 2015 Mar 1;23(1):65-8.
40. Kane MA, Bloch EM, Bruhn R, Kaidarova Z, Murphy EL. Demographic determinants of syphilis seroprevalence among US blood donors, 2011–2012. *BMC infectious diseases.* 2015 Dec;15(1):1-9.
41. Maan MA, Hussain F, Iqbal J, Akhtar SJ. Sexually transmitted infections in Pakistan. *Annals of Saudi medicine.* 2011 May;31(3):263-9.
42. Raza MH. HIV/AIDS and syphilis screening among high risk groups. *Journal of Rawalpindi Medical College.* 2015 Mar 30;19(1):11-4.