Original Research Article

ASSESSMENT OF THE PREVALENCE OF *Chlamydia trachomatis* INFECTION IN DONATED BLOOD AT REGIONAL BLOOD TRANSFUSION CENTRE, ELDORET, KENYA.

ABSTRACT

Aim: To detect *Chlamydia trachomatis* in donated blood at Regional Blood Transfusion center, Eldoret.

Methodology: A random selection blood sample was done for this study for a period of one month from July 24th 2017 to August 24th 2017. Blood bags were numbered 1-87 and picked amongst unnumbered blood bags by each donor in order of donation. The samples obtained were subjected to centrifugation and serum used in the immunoassay test by using the *Chlamydia trachomatis* IgG/IgM Elisa test kit.

Result: This study showed that *C. trachomatis* was present in 8% (7 samples) of the blood samples analyzed and 92% were found to be negative.

Conclusion: With 8% of the samples testing positive for *Chlamydia trachomatis* this indicated that some of the blood donated to the Regional Transfusion Center is contaminated.

Keywords: Blood, *Chlamydia trachomatis,* Detection, Kenya, Transfusion

1. INTRODUCTION

Chlamydia is a genus of pathogenic bacteria that are obligate intracellular parasites. Chlamydia infections are the most common bacterial sexually transmitted diseases in humans and are the leading cause of infectious blindness worldwide [1]. *Chlamydia trachomatis*, commonly known as chlamydia, is one of four bacterial species in the genus Chlamydia.

C. trachomatis is globally a major cause for sexually transmitted diseases (STDs) including Chlamydia. Chlamydia is one of the most common sexually transmitted infections worldwide affecting about 4.2% of women and 2.7% of men [2]. *C. trachomatis* cause various infections that include trachoma (conjunctivitis which is the most reported reason for blindness globally), pneumonic infections, and genital infections in people of both sexes and conjunctivitis in newborns. It is estimated that there are over 50 million new cases of *C. trachomatis* infection annually [3]. However another study done in 2015 showed that about 61 million new cases occurred globally [4].

C. trachomatis causes various contaminations in donated blood. A study estimated that about 92 million blood donations are collected per year in different parts of the world [5]. In addition, WHO published that 39 countries in the world still do not routinely screen for transfusion-transmissible infections (TTIs) including HIV, Hepatitis B, Hepatitis C and syphilis [6]. About 47% of blood donations in low-income countries are screened in laboratories which do not have capacity for *C. trachomatis* contamination [7]. Blood transfusion centers are expected to provide blood that is free of

contaminants. However, without this there is likeliness of transmitting fatal agents like *C. trachomatis* that may result in deadly diseases that may be found in blood [8].

Weström [9] asserted that one of the factors that may contribute to unnoticed *C. trachomatis* contamination of donated blood is that its infections are to a bigger percentage asymptomatic. This has commonly been reported in both males and females with about 50% males and 75% females of those affected having no symptoms.

MATERIALS AND METHODS

1.1. Sample Collection

Blood samples used in the study were collected aseptically using venipuncture techniques by qualified personnel of the center following the Pendergraph procedure of phlebotomy [10].

Use of aseptic techniques was observed to preserve the integrity of the specimen. Blood samples obtained were labelled with the donors code for *C. trachomatis* test. The sample blood obtained was subjected to centrifugation to separate the serum from the other blood components. The serum samples were then refrigerated at 2^{o}-8ºC in RBTC Hematology Laboratory to be used in the immunoassay test by using the *Chlamydia trachomatis* IgG/IgM Elisa test kit manufactured by Vircell Company Spain to find out the presence of *C. trachomatis* in donated blood using the ELISA procedure as shown below.

Prevalence of *C. trachomatis* was also determined with the help of the demographic questionnaire that is mandatory to be filled by the donor before any blood donation. For this age, gender, marital status and level of education of the donor constituted the factors considered during computation of the prevalence.

1.2. Sample Processing

10ml of blood sample from each donor was centrifuged at 1600rpm for 15 minutes. This was done when clotting had occurred and within 1 hour after blood sample was drawn. Serum was then used for the indirect immunoassay test.

1.2.1. Indirect Immunoassay

Preparations and Assay Procedure

The washing solution was prepared in advance. This was done by using distilled water to fill 50 ml of 20x washing solution up to 1000ml after which the solution was warmed up to 37ºC in the washing concentrate during storage before diluting. Once diluted, it was stored at 4ºC.

The Indirect Immunoassay Procedure

The incubator was set at 37ºC and all the reagents brought to room temperature before use (approximately 1hour), without removing the plate from the bag.
The components were then shaken well and plates removed from the package. The numbers of wells to be employed were determined that is: four wells for the controls; two for the cut off serum and one each for the negative and positive sera. Wells not required for the test were returned to the pouch and sealed.
For IgG test, 100 µl of serum diluent **2** was added to all wells followed by 5 µl of each sample, 5 µl of positive control **3G**, 5 µl of cut off control **4G** (in duplicate) and 5 µl of negative control **5G** into the corresponding wells. The plates were then shaken in a plate shaker for 2 min in order to achieve a homogenous mixture of the reagents. A sealing sheet was then used to cover the wells and incubated at 37ºC for 45 min.
For IgM test, 25 µl of VIRCELL IgG sorbent was added to each of the required wells, except for the wells where controls will be dispensed. 5 µl of sample was added and then 75 µl of the serum diluent **2** to each well. Control wells were prepared by adding first 100 µl of the serum diluent **2** to each well

and then 5 µl of the positive control **3M**, 5 µl of the cut off control **4M** (in duplicate) and 5 µl of the negative control **5M** to the corresponding wells. This was followed by plate shaking in a plate shaker for 2 min in order to achieve a homogenous mixture of the reagents. A sealing sheet was then used to cover the wells and incubated at 37ºC for 45 min.

After incubation, the seal was removed, liquid aspirated from all wells and washed five times with 0.3 ml of washing solution **9** per well and any remaining liquid was drained off.100 µl of IgG conjugate solution **6G** or IgM conjugate solution **6M** was immediately added into each well and a sealing sheet used to cover followed by incubation at 37ºC for 30 minutes.

After 30mins the seal was removed, liquid aspirated from all wells and washed five times with 0.3 ml of washing solution **9** per well. Any remaining liquid was drained off and 100 µl of substrate solution **7** immediately added into each well followed by incubation at room temperature for 20 minutes protected from light. After incubation 50 µl of stopping solution **8** was immediately added into all wells and finally readings were carried out within 1 hour of stopping.

2. RESULTS

In this study, a greater percentage of the samples were from the donors between 20-29 years of age (60.9%), while the least were below 20 years old group. There was a higher proportion of females (55.2%) than males (44.8%). Samples from single donors were 64.4% while divorced/separated and widowed were both 3.4%. 75% of the samples were from donors who were currently at tertiary education level while none was from those who had never attained any form of education. The highest proportion of the samples was from unemployed donors (73.6%), 11.5% from self-employed and 14.5% from employed persons (Table 1).

The largest percentage of samples were from donors who felt they were currently in good health (97.7%), had no sexual encounters with persons whom they did not know their background (96.6%), had irregular sexual partners (85.1).

Table 1. Overall characteristics of blood donors whose samples were analyzed at RBTC, Eldoret.

Characteristics	Categories	Total (N=87) No (%)
Age groups	Below 20 years	4 (4.6)
	20-29 years	53(60.9)
	30-39 years	25(28.7)
	40 and above	5(5.7)
Gender	Male	39(44.8)
	Female	48(55.2)
Marital status	Single	56(64.4)
	Married	25(28.7)
	Divorced/Separated	3(3.4)
	Widowed	3(3.4)
Education level	None	0(0)
	Primary	7(8.0)
	Secondary	14(16.1)
	Tertiary	66(75.9)

Employment status	Unemployed	64(73.6)
	Self-employed	10(11.5)
	Employed	13(14.9)
Good health	Yes	85(97.7)
	No	2(2.3)
Sexual activity	Yes	3(3.4)
	No	84(96.6)
Irregular sexual partners	No	13(14.9)
	Yes	74(85.1)

Detection of *Chlamydia trachomatis* in donated blood using IgG/IgM Elisa kit

Samples with indexes below 9 were considered as not having IgG or IgM specific antibodies against *C. trachomatis* while samples with indexes above 11 were considered as having IgG or IgM specific antibodies against *C. trachomatis*. Therefore this study showed that *C. trachomatis* in blood donors' samples at RBTC was found to be present in 8% (7 samples) of the blood samples analyzed at RBTC and 92% were found to be negative (Table 2).

Table 2: Antibodies Present in donor samples indicating *Chlamydia trachomatis* infection

Week	Donor No.	Antibodies against *C. trachomatis* IgM Antibodies	IgG Antibodies
1	1-25	-	-
2	32	-	+
	37	-	+
	41	+	+
	48	+	-
3	53	+	-
	59	-	+
4	86	-	+

- =Absence of antibodies, + = Presence of antibodies

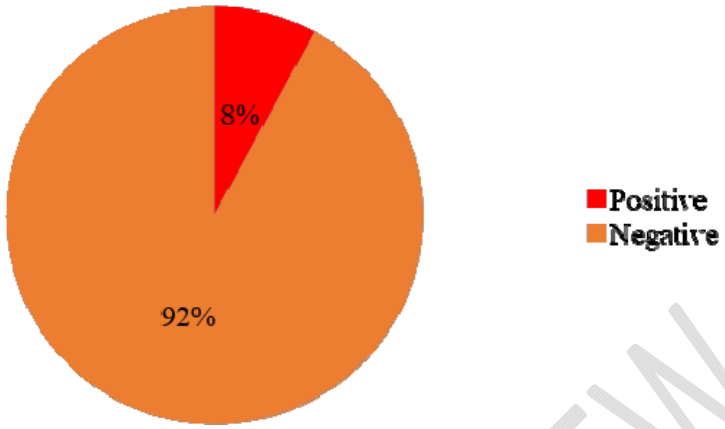

Figure 1: Proportion of donors' samples with Chlamydia trachomatis at RBTC

Associations Between *C. trachomatis* and Selected Variables

With the significance level at 0.05 the statistics revealed that having irregular sexual partners dependency with occurrence of *C. trachomatis* X^2 (1) = 4.667, p=0.031 but not significant. (Table 3)

Table 3: Detection of C. trachomatis infection in donated blood with respect to risk factors at RBTC, Kenya.

Characteristics		*C. trachomatis* status Positive N=7 Out of 87 samples	Chi Square tests Pearson Chi-Square	Valid cases	Asymp. Sig. (2-sided)
Age groups	Below 30 years	4	0.236	87	0.627
	30 years and above	3			
Gender	Male	2	0.706	87	0.401
	Female	5			
Marital status	Single	4	0.173	87	0.677
	Non-singles	3			
Education level	Primary and below	1	0.401	87	0.527
	Secondary and above	6			
Employment status	Unemployed	4	1.055	87	0.304
	Self-employed and/	3			

	Employed					
Irregular sexual partners	No	3	4.667	87	**0.031***	
	Yes	4				

Note: Non-singles include married, divorced, separated or widowed

* Significant at p<0.05.

4. DISCUSSION

The presence of *C. trachomatis* in blood samples was determined based upon the reaction of antibodies in the donor sample tested with the antigen adsorbed on the polystyrene surface. Unbound immunoglobulins were washed off, this was then followed by an enzyme anti-human globulin binding the antigen-antibody complex in a second step. After a new washing step, bound conjugate was developed with the aid of a substrate solution (TMB) to render a blue colored soluble product which turned into yellow after adding the acid stopping solution [11]. IgG/IgM antibody detection is a marker for a Chlamydia-positive immune response, either for current, chronic or past infections. The assay used in this study was the COMP (Complexes of Outer Membrane Proteins) of *C. trachomatis*, free from LPS which is responsible for most cross-reaction with other Chlamydia species. ELISA yields very good agreement with the target results (IgM100%, IgG 98%) in quality assessment schemes [12]. It provides good agreement with characterized samples from quality assessment schemes of IgM 100%, IgG 98% [12].Therefore ELISA technique is paramount for detection of *C. trachomatis* antibodies.

Occurrence of IgM antibodies without IgG being present is an indication of primary infection while presence of IgG antibodies without IgM antibodies in the blood sample is an indication of re-infection or presence of previous infections since IgG antibodies persists in the body for a long period of time. Presence of both IgG and IgM antibodies in the sample shows an ongoing infection.

In a study from Makkah Saudi Arabia, 8.7% of the women were positive for IgG antibodies to *C. trachomatis* with antibody indexes of 1.4−2.0 [13]. Low prevalence in Saudi patient population may be due to the adherence of strict moral principles and code of ethics in Saudi Arabia. In comparison to this study, 71.4% of women were positive for both IgG/IgM antibodies which is much higher.

Similarly, it has been shown in the USA and Europe that demographic factors which increase the risk of chlamydial infection include youth, single marital status and multiple sexual partners [14]. Therefore, a closer attempt should be made to correlate risk factors and disease entity when screening for *C. trachomatis*.

According to a study done by CDC,[15] on the prevalence of *C. trachomatis* among the adults, the highest rates is between 20 to 24 years old, however the age-specific rates for women was as low as 16 to 26 years old. This current study however slightly differs from the statistics by CDC. The age between 20 to 29 years of age showed the highest prevalence.

Multiple partnerships may increase the likelihood of encountering a sexually transmitted pathogen through the increased probability of choosing a partner with infection, while having new or casual sexual contacts may be related to increased risk because of a reduced familiarity between partners [16]. Statistics from this study revealed that having irregular sexual partners might have had dependency with occurrence of *C. trachomatis* X^2 (1) = 4.667, p=0.031.Therefore this study concurs with the later however it is not conclusive as per the statistics on the *chi square* on table 3.

Younger age has shown consistently to be associated with increased risk of chlamydial infection among the sexually active population with the highest incidence rates of infection being reported consistently in adolescents and young adults in Canada and the United States [17].This study

confirms the same as the highest prevalence is between the ages of 20 to 29. There are a number of reasons why adolescents are at greater risk for genital chlamydial infection than older people. A higher risk in adolescent females may be associated with certain aspects of physical development that make this group more vulnerable to sexually transmitted infections, including the persistence of columnar epithelium on the cervix, which supports the growth of *C trachomatis,* and changes in vaginal flora and mucus production [18]. As well, the older may have acquired partial immunity after initial or serial infections in the past [18].

5. CONCLUSION

The findings of this study therefore indicates that some blood donated to the Regional Transfusion Center was contaminated with *Chlamydia trachomatis* with 8% of the total samples indicating positive for *C. trachomatis* infection while 92 % were negative. Moreover the age group between 20 to 29 years old had the greatest percentage of those with *C. trachomatis*. Having multiple irregular number of sexual partners increases the chances of having *Chlamydia trachomatis* infection. However this is not be conclusive according to the statistics of this study.

The sex partners of donors with Chlamydial infection should therefore be referred for medical care through the efforts of individual donors. A further study is also recommended on the exact level of *C. trachomatis* by use of absorbance ranges.

6. COMPETING INTERESTS

"The authors declares that they have no competing interests"

7. ACKNOWLEGDMENTS

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8. AUTHORS' CONTRIBUTIONS

The authors' responsibilities were as follows; CP was author of the script, RF was the first supervisor while FK was the second supervisor.

REFERENCES

[1]. Ryan KJ, Ray CG. Sherris Medical Microbiology (4th Ed.). McGraw Hill. pp. 463–70; 2004.

[2]. Newman L, Rowley J, Vander Hoorn S, Wijesooriya NS, Unemo M, Low N, Temmerman M. Global estimates of the prevalence and incidence of four curable sexually transmitted infections in 2012 based on systematic review and global reporting. *PloS one*, (2015)*10*(12).

[3]. Centers for Disease Control and Prevention. Sexually Transmitted Disease Treatment Guidelines. *2003;* 51(6):1-78.

[4]. Global Burden of Disease. "Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015". *Lancet*. 2015; 388 (10053).

[5]. World Health Organization. WHO Global Data Base on Blood Safety, Summary Report. Geneva. 2011.

[6]. World Health Organization. Aide-memoire for national blood programmes. Geneva, Switzerland:http://www.who.int/bloodsafety/transfusion_services/en/Blood_Safety_Eng.pdf World Health Organization. Global database on blood safety: report 2001--2002. Available at http://www.who.int/bloodsafety/GDBS_Report_2001-2002.pdf. 2002.

[7]. World Health Organization. Prevalence and incidence of selected sexually transmitted infections; *Chlamydia trachomatis, Neisseria gonorrhoeae, syphilis and Trichomonas vaginalis*. 2008. Available at http://www.who.int/reproductivehealth/publications/rtis/9789241502450/en/

[8]. Mudasaar M. Transfusion-transmitted diseases. Department of Internal Medicine, Division of Geriatrics, Duke University Health System: 2008;10.1046/j.1537-2995.2000.40030335. Available at http://ihi.eprints.org/1702/1/Malimu%2C_Emmanuel_Zephania.pdf

[9]. Weström LARS, Joesoef, Reynolds GLADYS, Hagdu A, Thompson SE. Pelvic inflammatory disease and fertility. *Sexually transmitted diseases*, 1999; *19*(4), 185-192.

[10]. Pendergraph GE, Pendergraph CB. Handbook of phlebotomy and patient service techniques. Lippincott Williams, Wilkins.1998

[11]. Bas S, Muzzin P, Ninet B, Bornand JE, Scieux C, Vischer TL. Chlamydial serology: comparative diagnostic value of immunoblotting, microimmunofluorescence test, and immunoassays using different recombinant proteins as antigens. *Journal of clinical microbiology*, 2001; *39*(4), 1368-1377.

[12]. Gosink J. Serological Diagnostic for Chlamydia antibodies Infections. International Journal of Medical Microbiology.2015; *293* (148).

[13]. Ghazi HO, Daghestani MH, Mohamed MF. Seropositivity of *chlamydia trachomatis* among Saudi pregnant women in Makkah, J Family Community Med 2006; 13 (2):61–4.

[14]. Pramanik –Mania J, Kerkar S, Sonawane S, Mehta P, Salvi, V. Current Chlamydia trachomatis infection, a major cause of infertility. Journal of reproduction & infertility. 2012; *13*(4) 204.

[15]. Centers for Disease Control and Prevention. Atlanta, GA: US Department of Health and Human Services.2005.

[16]. Vranic SM. Chlamydia trachomatis Infections of the Adults. In Sexually Transmitted Infections. InTech.2012.

[17]. Division of STD Prevention. Sexually Transmitted Disease Surveillance, 2001. Atlanta: Centers for Disease Control and Prevention, 2002.

[18]. Navarro C, Jolly A, Nair R, Chen Y. Risk factors for genital chlamydial infection. Can J Infect Dis 2002; 13(3):195-207.

MINISTRY OF MEDICAL SERVICES

April 30, 2017

Patricia Chemutai

University of Eastern Africa Baraton

Department of Biological science/agriculture

Dear Patricia,

REF: APPROVAL TO COLLECT DATA AT THE REGIONAL BLOOD TRANSFUSION CENTRE.

Your clearance to collect data for research at the Regional Blood Transfusion Centre been from July 24th July 2017 to 30th July 2017 has been approved

Sincerely yours,

Thomas Rotich

The Kenya National Blood Transfusion Service

IT'S SAFE AND SAVES LIFE