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Prevalence of Human T-Lymphotropic Viruses Among Saudi Arabia Eastern Province Blood Donors: Twelve Years' Experience in the Blood Bank at King Fahd Hospital of the University

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Research Article

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Abstract

Objectives: This study aimed to examine the prevalence of Human T-cell lymphotropic virus type I and II (HTLV-I & II) and the cruciality of donated blood before transfusion in the blood banks in the Eastern Province of Kingdom of Saudi Arabia (KSA).

Methods: This retrospective cross-sectional study collected data from all blood donors over the period of 12 years (January 2008 to January 2020) from the blood bank database of King Fahad Hospital of the University (KFHU), Al Khobar, Eastern Province, Saudi Arabia, with the approval from Institutional Review Board, KFUH. The chemiluminescent micro-particle immunoassay (CMIA) has been the screening test and the Western Blot (WB) is the confirmatory test.

Result: 55998 blood donor files were analyzed. The prevalence was 0.001% and sensitivity and specificity for CMIA screening test compared to standard test WB was 100% and 99.8% respectively. Difference in the prevalence of HTLV-I & II in donated blood between the two study groups using the CMIA test was negligible. A statistically significant difference ($P<0.0001$) was demonstrated with western blot.

Conclusion: This study detects a low prevalence of HTLV-I/II infection among blood donors and a high sensitivity and specificity in the CMIA screening test. This urges the requirement of revised public health policies.

Keywords: Blood Bank; Human T-Lymphotropic Viruses; Prevalence; Saudi Arabia

Abbreviations: HTLV-I: Human T-cell Lymphotropic Virus Type I; TP: Treponema Pallidum; KFHU: King Fahad Hospital of the University; AABB: American Association of Blood Banks; ATLL; Adult T-cell Leukemia/Lymphoma.

Introduction

Human T-cell Lymphotropic virus type I (HTLV-I) was the first oncogenic delta human retrovirus isolated from the cultured cells of an African American patient with cutaneous T-cell lymphoma (mycosis fungoides) in 1980 [1]. Two years later, Human T-cell Lymphotropic virus type II HTLV-II) was isolated from a known case of hairy cell leukemia [2]. Both viruses are typical c-type retrovirus and belong to Ortho-retrovirinae subfamily [3]. HTLV-I prefers CD4+ve T-lymphocytes, but it was also detected in CD8+ve T-lymphocytes, dendritic cells, monocytes/macrophage, and epithelial cells [4]. HTLV-II prefers CD8+ve T-lymphocytes but it was detected also in CD4+ve T-lymphocytes, B lymphocytes, macrophages, and NK cells [5].

The mechanisms of cell specificity of HTLV-I and HTLV-II remain unclear till now and replication abilities are poor in non-T-lymphocytes for unknown reasons [6-8]. HTLV-I/II viruses need the cell-to-cell contact to cause infection. The cell entry is followed by the release of viral enzymes and 2 copies of single-stranded positive polarity RNA (ssRNA genome which undergoes reverse transcription into ssDNA that replicates to form double-stranded pro-viral DNA (dsDNA) [9]. The first oncogenic trans activator "Tax protein" generates leukemogenesis and pathogenesis foundation through activation of the cell cycle and growth factors [10]. A higher aligned nucleoprotein intasome formed by retroviral integrase to catalyze assimilation reactions has still an unclear role of host factor [11]. HTLV-I may be pathogenic in less than 5 % of infected persons. About 3-5 % of infected persons may develop a malignant disease of T lymphocytes known as Adult T-cell Leukemia/Lymphoma (ATLL) [12]. About 2-3% may develop HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP). Other inflammatory disorders like infective dermatitis, arthropathy, and uveitis may associate HTLV-I infection [13]. HTLV-II infection is associated with a few cases of hairy cell leukemia, neurodegenerative disorders, and chronic pulmonary infections [14]. Recent studies stated that there is no conclusive evidence that HTLV II is an aetiological agent of any specific disease.

Furthermore, the association between HTLV-II and leukaemia has not been confirmed yet and increased risk in HTLV-II patients with STAT3 mutation represents an increased risk of lymphoproliferative disease [15,16]. HTLV-I/II could be transmitted through white cell-containing blood components transfusion, needle injections, sexual intercourse, or by breast feeding [17]. The global estimated risk of transmission of HTLV-I/II virus through infected blood is ranging from 0.5% to 30% [18,19]. There is a 40% to 60% probability of seroconversion within 51 days following the transfusion of infected blood [20]. Some theories suggested that the transmission of HTLV-I/II needs the presence of living WBCs in the transfused unit. This was supported by the fact that the stored units for 7 days or more have a low chance to transmit the virus [21].

HTLV I/II screening is compulsory in the USA, Canada, Japan, Portugal, Greece, and Iran, however, it is still under debate in other countries due to their low prevalence of HTLV I/II infection as well as the high cost of these screening tests [22,23]. However, in some countries, like Sweden, performing these screening tests is mandatory only for the first time of donation [24]. Proper history taking, physical examination, screening tests, and leukodepletion are common strategies in the blood banks to avoid transmission of HTLV-I/II or other microbes through blood transfusion.

American Association of Blood Banks (AABB) standards and Federal regulations are strictly applied at King Fahad Hospital of the University (KFHU) blood bank. In 1994 and 1995, HTLV-I/II screening was started to be applied as blood donor serological screening in the Kingdom of Saudi Arabia (KSA) [25,26].

Since then, the screening of HTLV-I/II has been obligatory. All positive samples are rechecked in duplicates, and all double-positive samples are subjected to the confirmatory test. All previous studies in Saudi Arabia showed variable results for HTLV-I/II prevalence ranging from 0.000% – 0.0062% with relatively higher prevalence in the Eastern Province [27,28].

Therefore, this study aimed to examine the prevalence of HTLV-I/II viruses among blood donors in the Eastern Province of Saudi Arabia, to provide evidence about the implication of including HTLV-I/II screening tests and/or confirmatory tests and its cost-effectiveness in routine blood bank examinations for all donated blood in the Eastern Province.

Methods

Study Setting

This retrospective cross-sectional study collected data from all blood donors over the period of 12 years (January 2008 to January 2020) from the blood bank database of King Fahad Hospital of the University (KFHU), Al Khobar, Eastern Province, Saudi Arabia. The study was conducted after approval from Institutional Review Board, KFHU.

Study Protocol

The data were collected from all logbooks of serological positive tests, all logbooks of cases referred from KFHU to the "Infection Control Centre", all confirmatory reports done at the "Dammam Regional Laboratory", and all related reports received from the "Regional Centre of Preventive Medicine" in Al-Khobar, Eastern Province, Saudi Arabia.

All donors' charts were reviewed for retrieving data concerning the results of screening tests for HBV, HCV, HIV-I/II, HTLV-I/II, Malaria, and Syphilis infections, results of confirmatory tests for HBV, HCV, HIV-I/II, HTLV-I/II, Malaria and Syphilis infections, donor's nationality (either Saudi or non-Saudi). All donated blood units were tested for common transfusion transmitted microbes like HBV, HCV, HIV-I/II, HTLV-I/II, Malaria parasites, and Treponema Pallidum (TP) spirochetes by performing both screening and confirmatory tests.

All reactive samples by preliminary screening tests were rechecked again in duplicates. When one or both duplicates are still reactive by screening tests, the samples were submitted to the confirmatory tests. For HTLV-I/II infection, the CMIA-reactive rechecked samples were referred from the KFHU to the Dammam Regional Laboratory for confirmation by Western Blot.

Statistical Analysis

The univariate data analysis like frequency and percentages was used to describe the demographic and other categorical data in the sample. McNemar test was used to find the statistically significant difference between the CMIA screening test and the WB confirmatory test. A p value <0.05 was considered to be statistically significant. The data was analyzed using IBM SPSS (Statistical Package for the Social Sciences, version 23, Inc., Chicago, IL, USA).

Results

In our retrospective study, we viewed all the archived files among a 12-years period of time and we discovered that 55998 blood donors had been presented to the blood bank of KFHU. According to their nationalities, the total donor group was subdivided into two groups; the Saudi donor's group that constituted 40123 donors (71.7%) and the non-Saudi donors' group that constituted 15875 donors (28.3%).

In Table 1, the HTLV-I/II sero-prevalence rates, detected by the screening CMIA tests, among Saudi and non-Saudi donors' groups are summarized. Out of the total 55998 donors, only 61 (0.108%) showed reactivity for HTLV-I/II antibodies, mean-while, among the 40123 Saudi donors' group only 37 (0.092%) showed reactivity for HTLV-I/II antibodies and among the 15875 non-Saudi donors' group only 24 (0.151%) showed reactivity for HTLV-I/II antibodies.

Donors' groups according to nationality	By HTLV-I/II screening CMIA tests	
	Reactive	Negative
Total donors 55998	61 (0.108%)	55937 (99.892%)
Saudi group 40123 (71.7%)	37 (0.092%)	40086 (99.908%)
Non-Saudi group 15875 (28.3%)	24 (0.151%)	15851 (99.849%)

Table 1: HTLV-I/II Sero-Prevalence Rates by Screening CMIA Tests Among Saudi and Non-Saudi Donors' Groups Presented to Blood Bank of KFHU.

Table 2 shows the HTLV-I/II sero-prevalence rates detected by the screening CMIA tests in comparison to those detected when the confirmatory Western Blot (WB) tests were added among Saudi and non-Saudi donors' groups presented to blood bank of KFHU. Out of the total donors reactive to HTLV-I/II screening CMIA tests (61) only one donor was confirmed to be positive by the WB test, therefore out of the total donors (55998), the sero-prevalence rate for HTLV-I/II infection by WB test was (0.0018%). Also, out of the Saudi donor's group reactive to HTLV-I/II screening CMIA tests (37), none of them was confirmed to be positive by the WB test. On the other hand, out of the non-Saudi donors' group reactive to HTLV-I/II screening CMIA tests (24), only one donor was confirmed to be positive by the WB test, therefore out of the total non-Saudi donors (15875), the sero-prevalence rate for HTLV-I/II infection by WB test was (0.0063%). The only non-Saudi donor, who was confirmed to be positive for HTLV-I/II infection by WB test, was a 40 years old Indian male without any known risk factors. Furthermore, among the Saudi donors only one showed an indeterminate result by WB test. Mean-while, out of the total donors reactive to HTLV-I/II screening CMIA tests (61) only 5 donors (4 Saudis and 1 non-Saudi) were unfortunately not checked by the WB test because their samples were insufficient or they did not respond in spite of that they were frequently contacted. Finally, Table 2 shows a statistically significant differences in results between the screening CMIA and confirmatory WB test methods among the total donors, the Saudi donors, and the non-Saudi donors' groups (p= 0.000000, p= 0.000000, & p= 0.0000003, respectively).

Donors' groups according to nationality	Reactive donors by HTLV-I/II screening CMIA test (N: 61)	By confirmatory Western Blot tests for HTLV-I/II (INNO-LIA)				p-value
		Positive	Negative	Indeterminate	Not checked	
Total donors 55998	61 (0.108%)	1 (0.0018%)	54 (0.096%)	1 (0.0018%)	5 (0.0089%)	0.000000

Saudi group 40123 (71.7%)	37 (0.092%)	Zero (0.00%)	32 (0.079%)	1 (0.0025%)	4 (0.0099%)	0.000000
Non-Saudi group 15875 (28.3%)	24 (0.151%)	1 (0.0063%)	22 (0.138%)	Zero (0.00%)	1 (0.0063%)	0.000003

Table 2: HTLV-I/II Sero-Prevalence Rates by Screening CMIA Tests in Comparison to those Detected when the Confirmatory Western Blot (WB) Tests Were Added Among Saudi and Non-Saudi Donors' Groups Presented to Blood Bank of KFHU.

In table 3, the sero-prevalence rates of other transfusion transmitted microbes are highlighted among the Saudi and non-Saudi donors' groups with reactive HTLV-I/II screening CMIA tests. Out of the Saudi donors reactive to HTLV-I/II screening CMIA tests (37), only two (5.4%) were also reactive to HBcAbs by screening CMIA tests, however they were negative to HBsAg by screening CMIA tests, negative to HBV infection by confirmatory Nucleic Acid Amplification Testing (NAT), and negative to HTLV-I/II infection by HTLV-I/II confirmatory WB test see later in table 4. Mean-while, the Saudi donors reactive to HTLV-I/II screening CMIA tests (37) were negative to all other routinely tested transfusion transmitted microbes like HIV-I/II, HCV, Syphilis, and Malaria.

On the other hand, out of the non-Saudi donors reactive to HTLV-I/II screening CMIA tests (24), only one (4.17%) was also reactive to HIV-I/II antibodies by screening CMIA test; however, this donor was negative to HIV-I/II infection by the confirmatory WB test. Also, he was negative to HTLV-I/II infection by confirmatory WB test see later in Table 4 as well as he was negative to other routinely tested transfusion transmitted microbes like HBV, HCV, Syphilis, and Malaria. Furthermore, out of the non-Saudi donors reactive to HTLV-I/II screening CMIA tests (24), only two (8.33%) were also reactive to HBcAbs by screening CMIA tests, however those donors were negative to HBsAg by screening CMIA tests, negative to HBV infection by the confirmatory NAT tests, and negative to other routinely tested transfusion transmitted microbes like HIV-I/II, HCV, Syphilis, and Malaria. However, among these two non-Saudi donors who were reactive to both HTLV-I/II and HBcAbs by screening CMIA tests, one was further confirmed to be reactive to HTLV-I/II by the confirmatory WB test see later in Table 4. Therefore, his reactivity to HBcAbs by screening CMIA test could be attributed to a cross reactivity between HTLV-I/II antibodies and HBcAbs. Table 3 also shows, out of the non-Saudi donors reactive to HTLV-I/II screening CMIA tests (24) only one donor (4.17%) was also reactive to TP (Treponema pallidum) antibodies by screening Rapid Plasma Regain test as well as reactive to Syphilis infection by the confirmatory Fluorescent Treponemal Antibody Absorption test (FTA-ABS). Therefore, his reactivity to HTLV-I/II screening CMIA test could be attributed to a cross reactivity between HTLV-I/II and TP antibodies. This donor was negative to other routinely tested transfusion transmitted microbes like HIV-I/II, HBV, HCV, and Malaria. Mean-while, all non-Saudi donors reactive to HTLV-I/II screening CMIA tests (24) were negative to HCV and Malaria infections.

Screening and Confirmatory Tests of Other Transfusion Transmitted Microbes		**Reactive total donors by HTLV-I/II screening CMIA test (n=61)**	**Reactive Saudi donors by HTLV-I/II screening CMIA test (n=37)**	**Reactive non-Saudi donors by HTLV-I/II screening CMIA test (n=24)**
HIV-I/II	Reactive screening test by CMIA for (HIV-I/II Abs) and/or positive by NAT	1 (1.63%)	Zero (0.00%)	1 (4.17%)
	Reactive by confirmatory WB test	Zero (0.00%)	Zero (0.00%)	Zero (0.00%)
HBV	Reactive screening test by CMIA for (HBcAbs)	4 (6.55%)	2 (5.4%)	2 (8.33%)
	Reactive screening test by CMIA for (HBsAg)	Zero (0.00%)	Zero (0.00%)	Zero (0.00%)
	Positive by confirmatory NAT	Zero (0.00%)	Zero (0.00%)	Zero (0.00%)

HCV	Reactive screening test by CMIA for (HCV Abs) and/or positive by NAT	Zero (0.00%)	Zero (0.00%)	Zero (0.00%)
	Reactive by confirmatory RIBA test and/or positive by NAT	Zero (0.00%)	Zero (0.00%)	Zero (0.00%)
Syphilis	Reactive by screening RPR test for (TP Abs)	1 (0.0163)	Zero (0.00%)	1 (4.17%)
	Positive by confirmatory test (FTA-ABS)	1 (1.63%)	Zero (0.00%)	1 (4.17%)
Malaria	Reactive by screening test (RDT)	Zero (0.00%)	Zero (0.00%)	Zero (0.00%)
	Positive by confirmatory morphological examination of the PBS	Zero (0.00%)	Zero (0.00%)	Zero (0.00%)

Table 3: Sero-Prevalence Rates of Other Transfusion Transmitted Microbes among Saudi and Non-Saudi Donors Groups with Reactive HTLV-I/II screening CMIA tests.

- CMIA= Chemiluminescent Micro-particle Immunoassay
- WB= Western-Blot technique
- NAT= Nucleic Acid Amplification Testing
- RIBA= Recombinant Immuno-Blott Assay
- RPR= Rapid Plasma Regain
- FTA-ABS= Fluorescent Treponemal Antibody Absorption test
- RDT= Rapid diagnostic Test
- PBS= Peripheral Blood Smears

Table 4 shows the sero-prevalence rates of other transfusion transmitted microbes among Saudi and non-Saudi donors' groups with reactive screening CMIA tests and positive confirmatory WB tests for HTLV-I/II. Among the Saudi donors no one showed reactive screening CMIA tests and positive confirmatory WB tests for HTLV-I/II. On the other hand, among the non-Saudi donors with reactive screening CMIA tests and positive confirmatory WB tests for HTLV-I/II, there was only one donor who was also reactive to HBcAbs by screening CMIA tests, but he was negative to HBsAg by screening CMIA tests and also negative to HBV infection by the confirmatory Nucleic Acid Amplification Testing (NAT). Furthermore, he was negative to other routinely tested transfusion transmitted microbes like HIV-I/II, HCV, Syphilis, and Malaria. Therefore, his reactivity to HBcAbs by screening CMIA test may be attributed to a cross reactivity between the HTLV-I/II antibodies and the HBcAbs.

Screening and confirmatory tests of other transfusion transmitted microbes Total donors (n=1)		**Donors groups with reactive screening CMIA tests and positive confirmatory WB tests for HTLV-I/II**		
		Saudi donors (n=0)	**non-Saudi donors (n=1)**	
HIV-I/II	Reactive screening test by CMIA for (HIV-I/II Abs) and/or positive by NAT	Zero	Zero	Zero
	Reactive by confirmatory WB test	Zero	Zero	Zero
HBV	Reactive screening test by CMIA for (HBcAbs)	1	Zero	1
	Reactive screening test by CMIA for (HBsAg)	Zero	Zero	Zero
	Positive by confirmatory NAT	Zero	Zero	Zero
HCV	Reactive screening test by CMIA for (HCV Abs) and/or positive by NAT	Zero	Zero	Zero
	Reactive by confirmatory RIBA test and/or positive by NAT	Zero	Zero	Zero

Syphilis	Reactive by screening RPR test for (TP Abs)	Zero	Zero	Zero
	Positive by confirmatory test (FTA-ABS)	Zero	Zero	Zero
Malaria	Reactive by screening test (RDT)	Zero	Zero	Zero
	Positive by confirmatory morphological examination of the PBS	Zero	Zero	Zero

Table 4: Sero-Prevalence of Other Transfusion Transmitted Microbes among Saudi and Non-Saudi Donor Groups with Reactive Screening CMIA Tests and Confirmatory Western Blot (WB) Tests for HTLV-I/II.

- CMIA= Chemiluminescent Micro-particle Immunoassay
- WB= Western-Blot technique
- NAT= Nucleic Acid Amplification Testing
- RIBA= Recombinant Immuno-Blott Assay
- RPR= Rapid Plasma Regain
- FTA-ABS= Fluorescent Treponemal Antibody Absorption test
- RDT= Rapid diagnostic Test
- PBS= Peripheral blood smears

Discussion

In this study, we aimed to estimate the prevalence rate of HTLV-I/II antibodies among the blood donors represented to the blood bank of KFHU (in Al-Khobar city) during the period of time lasting from January 2008 to January 2020. We reviewed 55998 files of these blood donors; of which 40123 (71.7%) were Saudis and 15875 (28.3%) were non-Saudis. Our results showed very low prevalence rate of HTLV-I/II antibodies among our total donor group. Only 61 donors (0.108%) showed reactivity for HTLV-I/II antibodies by screening CMIA tests; of which, only one donor (0.0018%) was confirmed to be "positive" by HTLV-I/II Western Blot (WB) test and another one (0.0018%) was considered to be "indeterminate" by HTLV-I/II WB test. The donor with positive HTLV-I/II WB result was an expatriate (from India), while, the donor with indeterminate WB result was from Saudi Arabia. In our study, the HTLV-I/II prevalence rate by WB among the Saudi donor group was zero.

Similar or very close to our study results, several local studies, with different sample volumes, have been previously performed in different cities among the Kingdom of Saudi Arabia. In Riyadh city, Arif and Ramia [29] and El-Hazmi [27] studied 5900 and 20423 Saudi donors, respectively, and both reported zero prevalence rates and zero indeterminate results by confirmatory HTLV-I/II WB test [29,30]. Also, in Jeddah city, Al-Jaouni [30] and Hindawi, et al. [28] studied 9949 and 51168 Saudi donors, respectively, and both reported zero prevalence rates by confirmatory HTLV-I/II WB test, however, Al-Jaouni [30] found (0.14%) indeterminate results [28,31]. Similarly, in Aseer, AlShehri [31] performed HTLV-I/II WB test on 4281 Saudi donors and reported also zero prevalence rates and zero indeterminate results [32].

On the other hand, some other local studies showed prevalence rates moderately higher than those of our study for HTLV-I/II antibodies by WB test among blood donors. In Riyadh city, Bernvil, et al. [32] and Balkhy, et al. [33] studied 102753 and 24654 donors, respectively, by HTLV-I/II Western Blot test and both reported prevalence rates (0.0038%) and (0.004%), respectively, in comparison to ours (0.0018%). Mean-while, Bernvil, et al. [32] and Balkhy, et al. [33] rates for indeterminate results by WB test were (0.045%) and (0.15%), respectively, in comparison to ours (0.0018%) [33,34]. In Hofuf city, Ul-Hassan, et al. [34] studied 47426 donors by HTLV-I/II WB and they reported considerably higher prevalence rates (0.0062%) in comparison to ours (0.0018%). Their rate for indeterminate results by WB test was also higher (0.0042%) in comparison to ours (0.0018%) [35].

Only two old studies showed prevalence rates prominently higher than those of our study for HTLV-I/II antibodies by WB test among blood donors. In Dammam city, Fathalla, et al. [35] studied 40013 donors and they reported prominently higher prevalence rates (0.022%) in comparison to ours (0.0018%). Their rate for indeterminate results by WB test was also higher (0.009%) in comparison to ours (0.0018%). 36 Also, in Al-khobar city, Taha, et al. [36] studied 23493 donors by HTLV-I/II WB and they reported prominently higher prevalence rates (0.05%) in comparison to ours (0.0018%). Their rate for indeterminate results by WB test was also higher (0.017%) in comparison to ours (0.0018%) [37]. In spite of being performed in our local area (or city) the discrepancy in prevalence rates between these two old studies and our present study may be mainly attributed to the considerable difference in their sample volumes and ours. Also, it may be attributed to the chronological reduction of HTLV-I/II prevalence noticed in Saudi Arabia as well as occurred in some other countries among the world. For example, the HTLV-I/II prevalence has been reduced in Canada from 0.0093% in 1990 to be 0.0011% in 2010, [38] in Chile from 0.73% in 1991 to be

0.24% in 2010, [39,40] and in Brazil from 0.6% in 2000 to be 0.1% in 2008 [41].

According to the degree of HTLV-I/II prevalence rate, the regions of the world are categorized into regions with high prevalence rate (0.5% to 20%) or "endemic", regions with medium prevalence rate (0.1% to <0.5%), and regions with low prevalence rate (<0.1%) [42]. In fact, on searching the whole literature and publications, we found, up to the best of our knowledge, only ten local studies, similar to ours, have been performed inside Saudi Arabia since 1997 until now. Five of these ten local studies reported (zero %) prevalence rate of HTLV-I/II infection among their studied blood donors, [28-32] three studies reported HTLV-I/II prevalence rates (0.004%), (0.004%), and (0.0062%), respectively, [33-35] and the (relatively) highest were only two studies that reported HTLV-I/II prevalence rates (0.022%) and (0.05%), respectively [36,37]. Therefore, as shown in results of our current study, as well as repeatedly in results of all previous local studies, the Kingdom of Saudi Arabia is considered a region of low or even very low HTLV-I/II prevalence rate among its blood donors. In comparison, other studies highlighted the HTLV-I/II prevalence rates among blood donors in other Arabic or Gulf countries. For example, the HTLV-I/II prevalence rate reached (0.009%) in Kuwait, [43] while, it was estimated to range from (0.77%) to (3%) in Iran, [44] and it was reported to be 0.001% in Palestine [45].

In this study, among our total donor group (55998), 61 donors (0.108%) were reactive for HTLV-I/II by the screening CMIA test method, while, only one donor (0.0018%) was reactive for HTLV-I/II by confirmatory WB test method. We found a statically significant difference between the results obtained by the screening CMIA tests and those obtained by the confirmatory WB tests for HTLV-I/II antibodies among our total donor group (p-value=0.000000). Synchronously, we found almost similar statically significant differences between the results obtained by the screening CMIA tests and those obtained by the confirmatory WB tests for HTLV-I/II antibodies among our Saudi donors as well as our non-Saudi donor groups (p-values= 0.000000, and 0.000003, respectively). These statistically significant differences highlight the importance of the complementary use of both the HTLV-I/II screening CMIA test method and the confirmatory WB test method. Furthermore, out of our 61 donors that were reactive by HTLV-I/II screening CMIA test method, 54 donors (88.5%) were negative by HTLV-I/II confirmatory WB test method. Therefore, a serious concern about this high frequency of false positive results should be considered and all positive results obtained by the HTLV-I/II screening CMIA test method should be submitted to the confirmatory WB test method to discriminate the true positive cases from the false positive ones. In other words, we believe that performing the WB test method, in addition to the CMIA test method, is mandatory to minimize the frequency of false-positive results that could be obtained by the later screening tests if performed alone.

These false positive results by HTLV-I/II screening CMIA test could be attributed to a cross reactivity that may occur due to the presence of concurrent infection like Syphilis [46]. This goes with our finding among results of our non-Saudi donor's group that showed one "Filipino" donor who was reactive by the screening CMIA test for HTLV-I/II antibodies, reactive by the screening Rapid Plasma Regain (RPR) test for Treponema pallidum (TP) antibodies, and positive by the confirmatory Fluorescent Treponemal Antibody Absorption (FTA-ABS) test.

Another example for the cross reactivity in our study was observed among the results of our non-Saudi donor's group in which two donors showed synchronous reactivity to both HBcAbs and HTLV-I/II antibodies by the screening CMIA tests in spite of their negativities to both HBsAg by screening CMIA test and HBV infection by confirmatory Nucleic Acid Amplification test (NAT). However, one of these two non-Saudi donors was confirmed to be reactive to HTLV-I/II Western Blot test, hence, his reactivity to HBcAbs by the screening CMIA test was mostly due to the cross reactivity between HTLV-I/II antibodies and HBcAbs. Taha, et al. [36] findings were close to our present study; they reported one donor who showed synchronous reactivity to HBsAg, HBcAbs and HTLV-I/II antibodies by the screening CMIA tests, however, he was negative to HTLV-I/II Western Blot test [37].

The false positive results by HTLV-I/II screening CMIA test can be also associated with a variety of medical conditions or events like recent immunizations (e.g., for Influenza or Hepatitis viruses), current viral infections, autoimmune diseases, liver diseases (e.g., cirrhosis), hyper-gammaglobulinemia, and multiple pregnancies [47].

On the other hand, in spite of some authors have been reported that the results obtained by HTLV-I/II WB test method were compatible with those obtained by PCR test method, [48] many "positive results" by HTLV-I/II CMIA test method were giving "indeterminate results" by HTLV-I/II WB test method. This could represent an important concern among the blood banks worldwide. In our present study we discovered four donors were initially giving indeterminate results by HTLV-I/II WB tests, then on re-checking after six weeks later, three of these four donors were giving negative results and only one donor was remaining with an indeterminate result. The possible justification for these HTLV-I/II WB indeterminate results could be based on few different reasons, but in the majority of the cases, the reasons remain mostly unknown and considered a matter

of debate. In rare to few cases, these indeterminate results have been associated to 1) A cross-reactivity to other known retroviruses or a novel virus, 2) Antibody responses to a malaria parasite with epitope homology to HTLV-I, 3) A defective HTLV-I or HTLV-II, and 4) A low copy numbers of prototypic HTLV-I in the affected patient yielding the indeterminate antibody response [48]. Some authors have shown that the probability of WB indeterminate results to give positivity by HTLV-I/II PCR may reach up to 13% [49]. So, further confirmation by HTLV-I/II PCR may be necessary for all HTLV-I/II WB indeterminate results as long as the significance of the later remains uncertain.

Interestingly, in view of the average cost of HTLV-I/II screening CMIA test (around US$ 7) and that of HTLV-I/II confirmatory WB test (around US$ 57), we can imagine that the approximate estimated total cost of these tests performed among the last 12 years for the total blood donors (55998) included in our present study could be around (US$ 395463 = SR 1,482,986). This roughly estimated total cost does not include any other hidden costs (like those related to the working staff, machines maintenance, machines depreciation, controls consumption, repeated tests…… etc.) or added costs (like those for HTLV-I/II PCR tests needed for HTLV-I/II WB indeterminate results). This means that a direct total cost of (US$ 32955 = SR 123582) is required per year to detect HTLV-I/II confirmed positive cases among the donors that represent per year to one blood donation center in a local area with an estimated HTLV-I/II prevalence rate about 0.0018% (ranges from 0.00% to 0.0063%). Therefore, in our opinion, from the cost/benefit relationship, this cost is considered very high in relation to the very low HTLV-I/II prevalence rate observed either in our present study (among our area) or in almost all the previously published local studies (among the Kingdom of Saudi Arabia) along the last 23 years. In an attempt to reduce the cost of HTLV-I/I screening/confirming protocol, Balkhy, et al. [33] suggested to pool the blood samples then run the PCR test and by default they found that the cost of pooling the PCR test was half that of the regular screening test [34]. Also, AlShehri [31] suggested to pool at least 4 blood samples during the initial HTLV-I/II screening test [32].

In a country like Sweden with a very low HTLV-I/II prevalence rate similar to Saudi Arabia, one Swedish study, with a reported HTLV-I/II prevalence rate (0.002%), found that the cost of screening all of the donors is 18 times higher than screening only the new ones and that the screening protocol was estimated to prevent one death in every 200 years at a minimum cost of 36 million US Dollars. Accordingly, the Swedish National Board of Health decided that only the first donation should be screened for HTLV-I/II virus [24].

Conclusion

In our current study we confirm the generally very low prevalence rate of HTLV-I/II infection (0.0018%) among blood donors represented along the last 12 years to our KFHU blood bank, Al-Khobar, Eastern province, closely to many previously published studies from other different districts in the Kingdom of Saudi Arabia. Interestingly, the HTLV-I/II prevalence rate was (zero %) among our Saudi donors, mean-while, it was only (0.0063%) among our non-Saudi ones.

Competing Interest

The author declares no competing interest.

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