Seroprevlance of Human T-cell lymphotropic virus types 1 & 2 among blood donors in Aldamazin, Blue Nile State, Sudan.

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Abstract

Background

This study was carried out during the period from October 2016 to May 2017.

Objective :The study aimed to determine seroprevlance of human T-cell lymphotropic virus types 1 & 2 among blood donors in Aldamazin, Blue Nile State, Sudan.

Material and methods: All serum samples were analyzed for HTLV-I/II IgG/ IgM using enzyme-linked immune-sorbent assay (ELISA) (MP Diagnostic GmbH, - Germany).and a positive samples confirmed by Western Blot kit using (Fujirebio INNO-LIA - Belgium).

Results : *In the present study 12 (6%) were positive of 200 blood donor sample using indirect ELISA test (IgG + IgM).Only 2 (1%) were confirmed positive when using WB test.*

Key words : T-cell lymphotropic virus (HTLV) and retrovirus

Introduction

Human T-cell lymphotropic virus (HTLV) was the first human retrovirus discovered. HTLV belongs to the Retroviridae family in the genus Delta retrovirus. Retroviruses are RNA viruses that use an enzyme called reverse transcriptase to produce DNA from RNA. The DNA is subsequently incorporated into the host's genome. HTLV predominantly affects T- Lymphocyte (Proietti. 2005). Prior to 1979, the isolation of retroviruses was possible only in nonhuman Primates; in fact, it was believed that human retroviruses did not exist. In 2005 in Retro virology, Gallo reflected about earlier concepts that supported .This belief. First, if human retroviruses did in fact exist, then why had they not yet been discovered? Second, the virus was easily detected in animals, and Therefore should have also been easily detectable in humans.Third, technical difficulties hampered efforts to grow primary human cells in the laboratory.

Finally, it was shown that the human complement lyses animal retroviruses in vitro, suggesting erroneously that humans were intrinsically protected from these viruses (*Endo K, et al., 2002*).

The clusters predominate in a same-latitude trend. Phylogeny and molecular epidemiology studies have been used to explain this behaviour of the infection. **In the United States**HTLV-1 infection is linked to immigrants, children of immigrants, sex workers, and injection drug users. Based on transfusion data from 2000-2009 among first-time donors, the prevalence of HTLV-1 was 5.1 cases per 100,000 population and was associated with female sex, older age, and black and Asian race/ethnicity. (Zunt, 2006).

HTLV-2 infection affects Native American Indians. Some tribes have seroprevalence rates as high as 13%.Intravenous drug users, in whom the seroprevalence is estimated to be about 20%, with a disproportionate share occurring in African American injection drug users. (Willems, 2009).

In Africa, the seroprevalence increases from the north to the south, varying from 0.6% in Morocco to greater than 5% in several sub-Saharan African countries, for example, Benin, Cameroon, and Guinea-Bissau. In Europe and North America, the prevalence is low and limited to groups that emigrated from areas of endemicity. For blood donors, very low rates were found in France (0.0039%) and the United States (0.025%) (Miyamura, 2009).

In South America, the virus was found in all countries. Medium prevalence was found in blood donors from Chile (0.73%) and Argentina (0.07%) (Brites ,2009).

Objective : To determine seropositive of human T-cell lymphotropic virus types 1 & 2 among blood donors in *Aldamazin*, Blue Nile State, Sudan.

Materials and Methods

Study design:

This is an analytic prospective study, designed for the seroprevalence of HTLV-1 & 2 infection among Blood Donors in Blue Nile State, Eastern Southern Sudan.

Study Area:

The study was conducted at Aldamazin town, the capital of the Blue Nile State which is located 525 Km south of Khartoum the capital of Sudan. The state extends from Sennar State in the north, bordering Ethiopia in the east and South Sudan into the west and south. It is an agricultural and postural state. The population of this state is 861000 persons .Most of them are farmers and animal breeders.

Study population:

Adult blood Donors attending Central Blood Bank in *Aldamazin* Teaching Hospital.

Study period:

This study was conducted during the period from October 2016 to May 2017.

Ethical Clearance: Written informed consent was given to participants.

Sample size:

Two hundred blood samples (n=200) were collected from blood donor using the following equation:

$$N = \frac{(1.96)^2 P(1-p)}{d^2}$$

N=population size
Z=critical value of the normal distribution at the required confidence level
P=sample proportion
D=margin of error

Sample processing:

Collected blood samples were tested for the presence of anti-HTLV I/II IgM and IgG using the commercially available ELISA kits (MP Diagnostic GmbH, - Germany). Western Blot kit (Fujirebio INNO-LIA - Belgium).

Inclusion Criteria:

Male and female attended to the Central Blood Bank in Aldamazin Teaching Hospital during the study period such as blood donor.

Exclusion criteria:

Blood donors younger than 18 years old or elder than 55 years old were excluded from this study.

Sample Collection:

Five ml of blood was collected in a plain container by venipuncture using sterile syringe. Each sample was centrifuged at 3000 rpm for 15 min. The serum was collected in 2 ml sterile plain container, labelled and placed in a plastic rack. Samples were kept frozen at-20˚ C until tests performed. One ml was used for performing ELISA test, the second ml was used for Western Blot test.

Test principle:

The wells of the polystyrene microplate strips are coated with mixture of three different HTLV recombinant proteins, which correspond to the highly antigenic segments of HTLV-1and HTLV-2 viruses. The conjugate is based on a tri-fusion recombinant protein, which is labelled with horseradish peroxidase. The tri-fusion antigen is

generated by cloning of three c DNA fragments coding for the three HTLV recombinant proteins into a single vector. Human sera or plasma, diluted in the diluent containing the conjugate, are incubated in these coated wells. HTLV1/2 specific antibodies (IgG and IgM) if present, will bind to both the antigens immobilised on the solid phase and the tri-fusion antigen of the conjugate. After incubation, the wells are thoroughly washed to remove unbound materials. Colourless substrate solution containing chromogen 3,3,5,5 – tetramethylhylbenzidine (TMB) is then added to each presence of a blue colour after incubation, which changes to sulphuric acid. The intensity of the resulting yellow product is proportional to the amount of antibodies present in the specimen.

Statistical analysis: All data were analysis using Statistical Package for Social Sciences (SPSS) soft-ware version (16) USA. We used in data analysis of different variables (ANOVA),Pearson chi-square test and paired samples test.

Results

Sex groups:

The study population according to sex group showed that 198 (99%) were males and 2.0 (1%) were females. (Fig. 1).

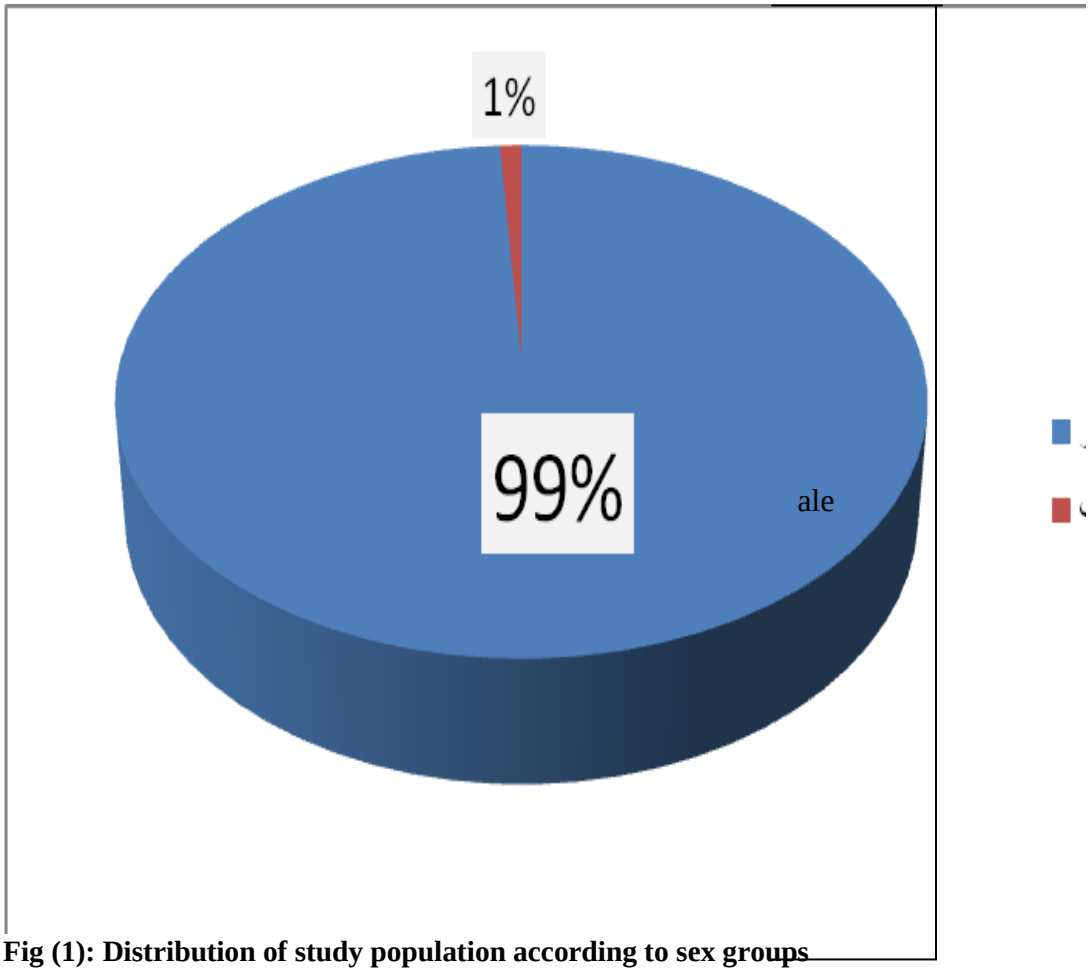

Fig (1): Distribution of study population according to sex groups

Age groups:

The study population according to age group showed that 32 (16 %) were 18-25 years old, 76(38 %) were26-33 years old , 42 (21 %) 34 - 40 years old, 29 (14.5) were 41 – 47 years Old and 21 (10.5) 48 – 55 years old.

Table (1) Distribution of study population according to age groups

Frequency	percent	Age group
32	16 %	18 – 25 Y
76	38 %	26 – 33 Y
42	21 %	34 – 40 Y
29	14.5 %	41 – 47 Y
21	10.5 %	48-55 Y
200	100 %	Total samples

Education: The educational level of the study population was 115(57.5) primary education, 42 (21 %) secondary education and 43 (21.5 %) university educations. (2)

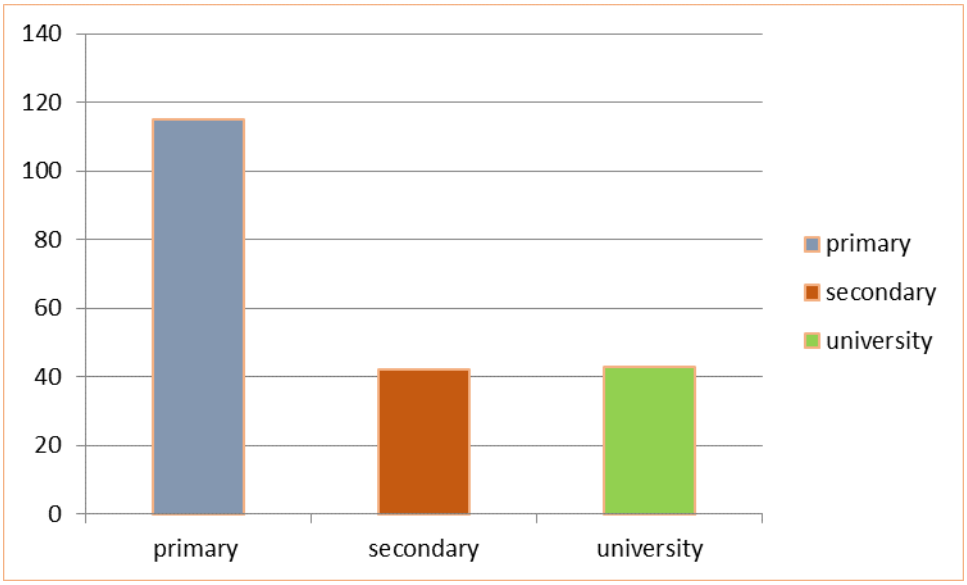

Fig (2) Distribution of study population according to education level .

Donation: In the study population, 93 (46.5 %) were donated for the first time, 107 (53.5%) Were Donated several time (Figure .3) .

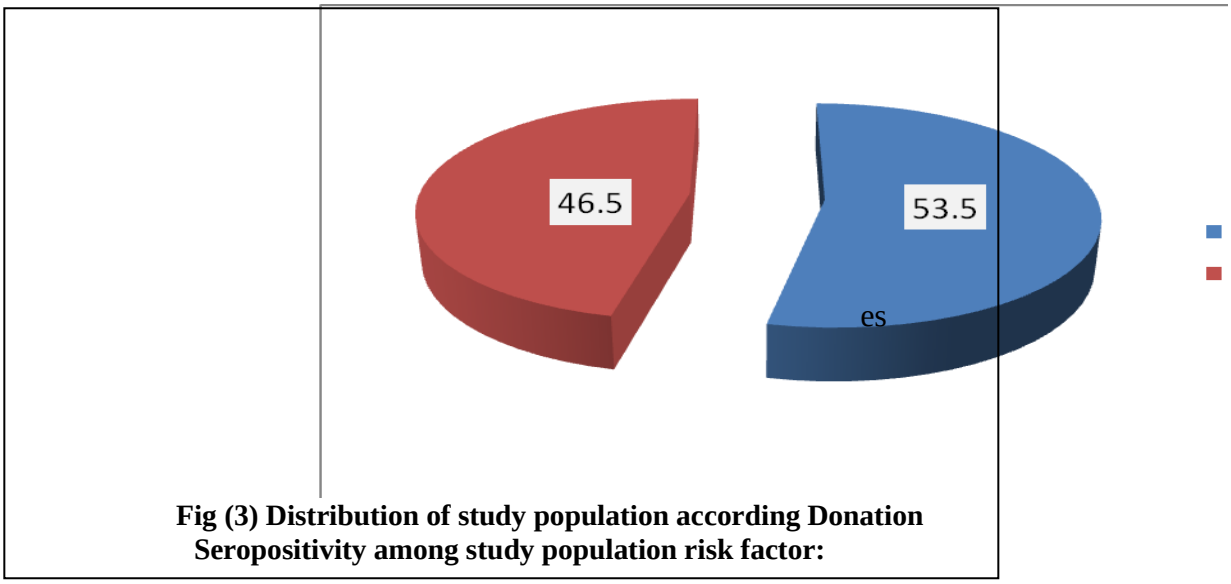

Fig (3) Distribution of study population according Donation Seropositivity among study population risk factor:

Serpositively of HTLV-I by ELISA: In the study population, 12 (6. %) had positive for HTLV-I and 188 (94 %) had negative. (Fig4).

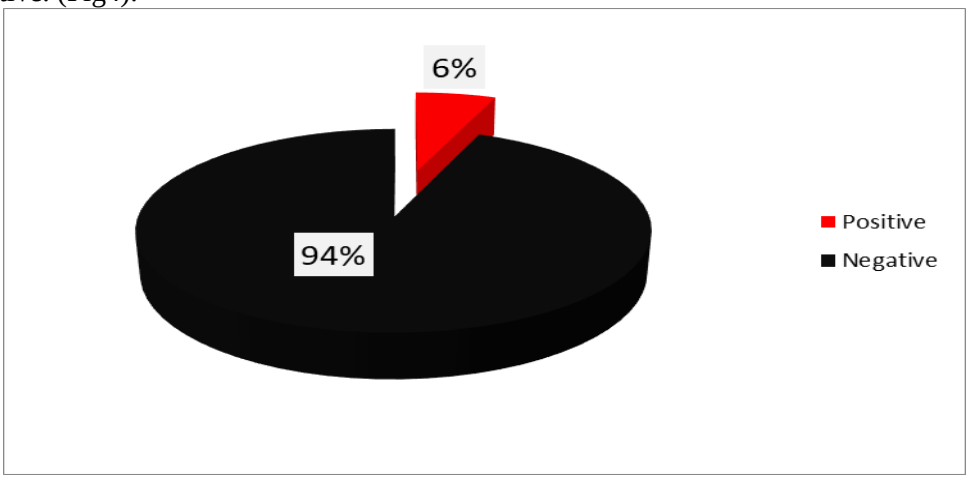

Fig (4): ELISA for HTLV-1 results among the study population

Serpositively of HTLV-II by ELISA:

In the study population, all blood samples give a negative result for HTLV-II. (Fig 5).

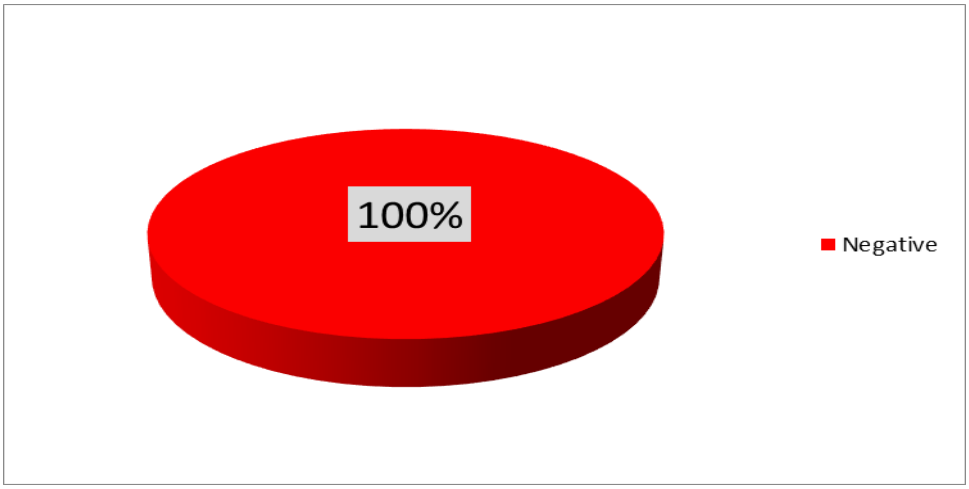

Fig (5): ELISA for HTLV-I1 results among the study population

Serpositively of Western Blot for a positive samples by ELISA:

The positive samples of HTLV-I were confirmed by WB test. The WB results indicate that only 2 (1 %) specimens were positive of HTLV-1 and 10 (5 %) specimens were negative (Figure 6) .

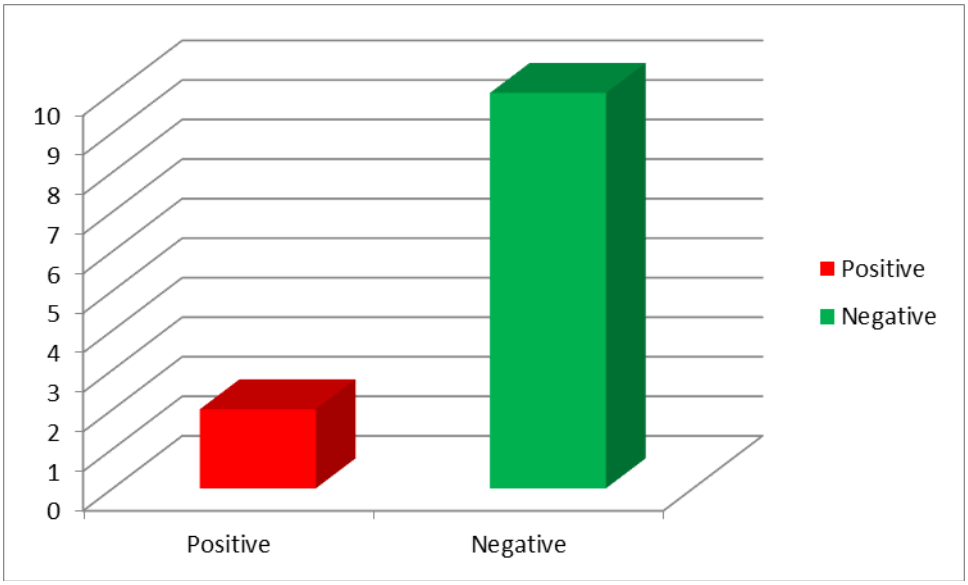

Fig 6- WB test for a positive HTLV-I sample by ELISA

None of the female donors was found to be seropositive for HTLV-1/2. All variables from the univariate analysis including age, sex, place of birth, occupation, and level of education were entered into multiple logistic regression models. In this analysis, no independent risk factors were found to be significantly associated with HTLV-1/2 seropositivity.

Discussion

The blood donors have been suggested as the best source of data for epidemiologic studies in Africa, but do not cover all demographic and socioeconomic strata. The majority of the blood centres are located in urban areas of big cities, and the blood donors generally are of good health, age restricted, and previously selected for behaviours that would put them at low risk for acquiring or transmitting infectious diseases. The prevalence of human T-cell lymphotropic virus (HTLV-1) in Sudan has been poorly documented.The transmission through blood transfusion is most efficient route for HTLV-I/II (Melo ,2000).

In the present study 12 (6%) were positive of 200 blood donor sample using indirect ELISA test (IgG + IgM).Only 2 (1%) were confirmed positive when using WB test. No previous study are available concerning the prevalence of HTLV in Sudan to compare with this study.

Our study finding (1%) which the same as that found in blood donor in Mozambique (0.9 %) using ELISA test and WB as confirmatory test (Fathalla ,1998) .

Also overall 1.0 % HTLV-1 seroprevalence in our study was nearly similar to that found in Mashhad (IRAN) blood donors (0.77 %). Alow prevalence rate was found in Saudi Arabian blood donors (0.022%), in United States (0.004%), in France (0.004%) and Brazil (0.42%) which is lower than our finding (1.0%). (Fathalla 1998).

This low rate may be due to the fact use more expensive and accurate tests. Higher seroprevalence rate concerning HTLV-1 in blood donors has been found in Jamaica (2.1%) and in Nigeria (3.6%) compare to our finding (1.0%).(Feuer,2005).This variation may be due to the use of ELISA test only without confirmatory test.

Conclusion

- we conclude that WB test was superior to indirect ELISA test.
- Apply WB tools as gold standard for the confirmation of HTLV infection.
  Recommendation**:** We recommended that :
- Western blot test should be used for confirmation of ELISA results.

- Specific programs strategies targeting HTLV vaccination should be developed.
- Further studies using large sample size should be recommended.

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