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PREVALENCE, SEROTYPING AND ASSOCIATED FACTORS OF HIV VIRUS AMONG AIDS PATIENTS

Microbiology

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ABSTRACT

Background: Yemen is classified as a low prevalence country with an estimated HIV prevalence at 0.3% in the general population. By the end of December 2018, a total of 10300 cases were reported to National AIDS Program.

Objective: This study aims to determine HIV typing among Yemeni AIDS patients, and the association of possible risk factors.

Methods*:* A cross-sectional study was conducted with 194 HIV-infected patients (134 males and 60 females) attending National AIDS Center at Aljumhory Educational Hospital, Sanaa, Yemen. Blood samples of HIV patients were collected, separated and tested for detection of HIV-1 and HIV-2 antibodies using commercially available kits of ELISA method.

Results: Higher incidence rate of HIV-1 among male was 0.28/1000, more than female 0.11/1000. There were no effect of blood transfusion and contracting HIV-2 (P = 0.6), history of surgical operation (P =0.25), dental procedure (P = 0.6), or with history of traveling abroad (P =0.06). Highly significant of possible associated factors were among family member suffering from AIDS infection contracting HIV-2 and history of injury (RR=2.1, P= 0.01), (RR=2.4, P= 0.007)

Conclusions: Majority of the HIV positive subjects were HIV-1 followed by HIV-2. Co-infection by HIV-1/HIV-2 may lead to antiretroviral resistance. HIV screening in Yemen should be test for co-infection; antiretroviral resistance.

KEYWORDS

Human Immunodeficiency Virus, Acquired Immune Deficiency Syndrome, Serotyping, Incidence

INTRODUCTION

Human Immunodeficiency Virus (HIV) is a Lentivirus that can lead to Acquired Immunodeficiency syndrome (AIDS). This is a condition in which the immune system of Human begins to fail which paves way for opportunistic infections to thrive. Despite recent scientific efforts made in the discoveries and advances in understanding and controlling the virus that causes AIDS, this progress has little impact on the majority of HIV infected people and population living in developing countries. The social and economic conditions that support the viral spread have to be addressed, as important elements in local and global efforts to curb its spread and create effective solution to the increasing trend of the epidemic especially in developing communities (Azuonwu *et al;* 2017, Obioma and Chikanka, 2018, UNAIDS 2018, UNAIDS 2019). There are two major types of the human immunodeficiency virus. HIV-1, which was discovered first, is the most widespread type worldwide. HIV-2 is more than 55% genetically different from HIV-1. Due to this genetic difference, HIV-1 and HIV-2 antigens are distinct enough that if a test is developed only to detect HIV-1, it will not reliably detect HIV-2. However, tests which are sensitive to both types of virus have been developed. Each major type of virus can be further broken down into groups, which themselves can be subdivided into clades or subtypes. HIV-1 comprises groups M (main), O (outlier), and N (non-M or O). There are two main HIV-2 subtypes, A and B. Issues to do with the transmission of HIV-2 and different HIV-1 subtypes are covered in previous sections. Screening tests in developed countries were originally developed to identify the most common HIV subtype in those regions group M, clade B (Sallam *et al;* (2017). In addition, third- and fourth-generation ELISA antibody tests are reliably able to detect group O virus, and the full range of group M subtypes. All the third-generation, fourth-generation and rapid tests are sensitive to HIV-2 antibodies (Parry *et al;* 2003, Dougan *et al;* 2005). Although treatments for AIDS and HIV is available for registered cases in Yemen which slow the course of the disease, there is no research program for control and prevention which is the key aim in controlling the AIDS in Yemen. This program should promoting safe sex, save blood transfusion and needle-exchange programs in attempts to prevent or at least slow down the spread of the virus among community. The aim of this study were carried out in order to determine the HIV typing among AIDS patients in Yemen and to associated of possible risk factors.

MATERIALS AND METHODS

Sample collection

A cross-sectional study was conducted with 194 HIV-infected patients attending National AIDS Center at Aljumhory educational hospital, Sanaa, Yemen. A brief clinical history, high risk behaviour and history of blood transfusion were noted and history of visits abroad. Samples of AIDS patients were collected, separated stored at - 20°C until use.

Experimental procedure

Enzyme linked immunoassays were performed with slightly modified according to Al-Haj *et al;* 2018a. Briefly, *500* ng peptide dissolved in *0.05* M carbonate bicarbonate buffer (pH 9.6) was immobilized in each of the wells of a q6-well microtiter plate (Falcon, Becton Dickinson labware, NJ, USA). Wells were washed with phosphate buffer containing *0.05* Tween-20 and blocked with buffer containing 2% bovine serum albumin fraction V (Sigma-Aldrich, St. Louis, MO, USA).in PBS-Tween for I hour at 37 C. After washing, 100 µ of the test and the control sera (diluted 1:300) were reacted with the peptide for 1 hour at 37 C. All the washes were carried out using an automated micro-plate washer (ELx 50, BIO-TEK instruments, Vermont, USA). Subsequently, *100* µl of tetra-methyl-benzidine (TMB)(Sigma-Aldrich, St. Louis, MO, USA) substrate was added; plate incubated at RT for 30 min and the reaction was stopped with 100 µl of 1 N H_2SO_4. The reading was taken at 450 nm wavelength with a reference at 630 nm using an automated micro-plate reader (ELx-800, BIO-TEK instruments, Vermont, USA). All the samples were tested in duplicate wells and the mean of their absorbance was taken as the final absorbance value.

Statistical analysis

Data was analyzed statistically using the Epi Info statistical program version 6 (CDC, Atlanta, USA) with minor modified according to (Nagi ALHaj, 2018,b and c). The Chi-square test was used to analysed, the qualitative data, p value < 0.05 was considered as significant and Odds ratio with 95% confidence interval was used to evaluate the association of various age groups with respect to AIDS patients of male/female.

RESULTS

Serotyping study was carried out for a total of 194 AIDS patients (134

males and 60 females). The age and sex distribution of the study group as shown in Table 1, and the main age male was higher (41 ± 9.6 years) than for female (38.4 ± 6 years) with no patents under 20 age.

Table 1: Age and sex distribution of HIV typing of AIDS patients.

Characters Age groups	Male (n=134)		Female (n=60)		Total n =194		χ2	p
	No.	%	No.	%	No.	%		
20-29 years	18	13.4	4	6.7	22	11.3	1.9	0.16
30 – 39 years	34	45.6	36	60	70	36.1	21.5	<0.01
40 – 49 years	60	44.8	16	26.7	76	39.2	5.7	0.01
≥ 50 years	22	16.4	4	6.7	26	13.4	3.4	0.06
Total	134	69.1	60	30.9	194	100		
Mean age	41 years		38.4 years		40.2 years			

χ2, Chi-square = (significant), p Probability value = 0.05 significant

Crude incidence rate for HIV-1 was 0.190/1000 and sex incidence rate for HIV-1 among male was 0.28/1000, higher than female 0.11/1000, while the relative risk for contracting HIV-1 was 2.5 times among male with CI=1.86–3.4 which were highly significant (X2 =37.9, P> 0.001). When age was considered, the highest incidence rate was occurred among age group 40-49 years old (0.373/1000), with RR= 2.6, CI=1.9 – 3.4, χ2 =44.6, PV<0.001followed with a high incidence rate among age group ≥ 50 years old (0.256/1000), RR= 1.4, CI=0.9 – 2.1 as shown in Table 2.

Table 2: HIV-1 among different sex, age groups and relative risk

Characters	Positive HIV (n =194)		RR	CI	χ2	P
	No.	/1000				
Sex Male (n=478111)	134	0.28	2.5	1.8-3.4	37.9	>0.001
Female (n=539145)	60	0.11	0.4	0.3-0.5	37.9	>0.001
Age groups 20-29 years (n=386557)	22	0.05	0.2	0.13-0.33	58.5	>0.001
30 -39 years (n=325504)	70	0.215	1.2	0.9-1.6	1.5	0.2
40 – 49 years (n=203440)	76	0.373	2.6	1.9-3.4	44.6	>0.001
> 50 yrs (n=101755)	26	0.256	1.4	0.9-2.1	2.5	0.11
Crude prevalence rate N=1017256	194	0.190				

RR Relative risk = > 1, **CI** Confidence intervals, χ^2, Chi-square = (significant), ***p*** Probability value = 0.05 significant

Prevalence rate of HIV-2 among AIDS patients was 22.7% and according to sex the male 20.9 %, lower than female 26.7% as shown in Table3.

Table 3: HIV-2 among different sex, age groups and relative risk

Characters	Positive HIV II (n = 44)		RR	CI	χ2	P
	No.	%				
Sex Male (n= 60)	28	20.9	0.8	0.5-1.3	0.7	0.3
Female (n= 134)	16	26.7	1.3	0.8-2.2	0.7	0.3
Age groups 20-29 years (n=22)	4	18.2	0.8	0.3-1.9	0.29	0.59
30 -39 years (n=70)	20	28.6	1.5	0.9-2.5	2.2	0.14
40 -49 years (n=76)	16	21.2	0.9	0.5-1.5	0.19	0.66
> 50 years (n=26)	4	15.4	0.65	0.25-1.6	0.9	0.3
Crude prevalence rate N=194	44	22.7				

RR Relative risk = > 1, **CI** Confidence intervals, χ^2, Chi-square = (significant), ***p*** Probability value = 0.05 significant

The relative risks (RR) were estimated and potential risk factors of contracting HIV-2 the study found no effect of blood transfusion, contracting HIV-2 (RR=1.3, CI=0.6 – 2, X^2=0.26 and P = 0.6), history of surgical operation (RR=1.4, CI=0.8 – 2.3, X^2 = 1.3 and P =0.25),dental procedure(RR=1.3, CI=0.5 – 3.2, X^2=0.3 and P = 0.6) and with history of traveling abroad (RR=1.6, CI=0.97– 2.7, X^2 = 3.3 and P =0.06). Family member suffering from AIDS infection and contracting HIV-2 and history of injury (RR=2.1, CI=1.2 – 3.7, X^2=5.2 P = 0.01), (RR=2.4, CI=1.2 –4.9, X^2=7.1 and P = 0.007) were the highly significant respectively Table 4.

Table 4: Relative risk and Potential risk factors of AIDS patients with HIV-2

Risk factors	Positive HIV II n=44		RR	CI	X2	PV
	No	%				
Blood transfusion n=126	30	23.8	1.3	0.6-2	0.26	0.6
Dental procedure n=172	40	23.3	1.3	0.5-3.2	0.3	0.6
Injury n=126	36	28.6	2.4	1.2-4.9	7.1	0.007
Family HIV n=24	10	41.7	2.1	1.2-3.7	5.6	0.01
Operation n=74	20	27	1.4	0.8-2.3	1.3	0.25
Traveling abroad n=58	18	31	1.6	0.97-2.7	3.3	0.06

RR Relative risk = > 1, **CI** Confidence intervals, χ^2, Chi-square = (significant), ***p*** Probability value = 0.05 significant

DISCUSSION

Yemen is classified as a low prevalence country with an estimated HIV prevalence at 0.3 % in the general population according to the last cases reported to National AIDS Program December 2018 with a total of 10300 cases. The majority of transmission is attributed to sexual transmission whether hetero- (62%) or homo- sexual (7% among new infections). Yemen is a part of MENA regions where the number of new infections is increasing although the principal routes of transmission vary from one country to another but the majority of infections occur among people who inject drugs and injecting partners and commercial sex networks. HIV in MENA is concentrated in certain groups with behaviours that put them at a higher risk of infection namely, men who have sex with men, female sex workers, and people who inject drugs (UNAIDS 2018, UNAIDS 2019). In this study the crude incidence rate of HIV-1 in adults over 20 years old individuals was 0.19/1000 for total population of Sana'a city, these lower than previous reported of different country such as Northern, Western, and Central Europe, the Northern Mediterranean, North America, and Australia, among general population where the rates of HIV was ranged from 0.5-2 %, and China, Southeast Asia, and tropical Africa among general population where the rates of HIV are ranged from 20-36 %, (Chu and Selwyn, 2008, Kallings, 2008). But not lower than previous reported in Eastern Europe, Russia and the Russian Federation, Southwest Asia, Central and South America among general population where the rates of HIV was ranged from 5-12 %, (Gallo, 2006). The present study show that the HIV infections have become more increasing reports in Yemen regardless the absence of measures to prevent transmission is carried out in spite of this increase. The data of the present study provide a strong evidence that the acquisition and transmission of HIV infection are enhanced in the presence sexual transmitted diseases. This led to recommendations that improved HIV treatment should form one component of HIV prevention and control programmes in Yemen. Therefore the possibility of further increase in the number of HIV cases in Yemen cannot be dismissed. The specific male incidence rate was 0.28/1000 higher than female rate 0.11/1000, with a highly statistically significant. The higher male rate was similar among sex distribution of HIV infection in Western Europe and the Mediterranean where the male higher rates of the distribution is the feature in all reports form theses regains (Sousa *et al;* 2010). The majority of transmission is attributed to sexual transmission whether hetero-(62%) or homo-sexual (7% among new infections). Although many reports showed that 34% are females and that around 81% of all the cases are aged 15-49 years. However, our higher male rate was different from the sex distribution of HIV infection in sub-Saharan Africa and the USA among black population where the female rates was twice of HIV and more than male rates (CDC-a 2003 and WHO 2011). HIV infection effects both adults and children everywhere (Kalish *et al*. 2005), but the drawback of this study because did not included AIDS patients below age 20. There was also trend toward increased levels of infection with the fourth decade of life where rate is equal to 0.373/1000 and highly significant relative risk for this group equal to 2.6 times than other groups (X^2=44.6, PV > 0.001). Previous report in Yemen had been mentioned similar finding among general population and risk groups which reported that higher rate of HIV were in the 4th decade. The increasing of incidence rate with increasing age in our study could be attributed to an accumulation risk of infection over time. Majority of AIDS patients get their infection with HIV through sexual transmission which occurs during direct sex practice or contact of secretions, genital or oral mucous membranes of another and/or injection drug (Cayley, 2004). Two types of HIV can be distinguished genetically and antigenically. The HIV-1 is the cause of the current

worldwide pandemic while HIV-2 is found in West Africa but rarely elsewhere. HIV-2 (Lakhanpal and Ram, 2008), which is transmitted in the same ways as HIV-1, causes AIDS much more slowly than HIV-1 but otherwise clinically the diseases are very similar. HIV-1 and HIV-2 are thought to have arisen from two natural hosts both harbouring simian immunodeficiency virus (SIV): HIV-1 resulted from human infection by simian immunodeficiency virus *SIVcpz* chimpanzees and HIV-2 resulted from infection by SIV-infected *sooty mangabeys* (Kalish *et al;* 2005). The prevalence of HIV-2 in Yemeni AIDS patients was 22.7% as co-infection with HIV-1. This result can be explained (co-infection) by the finding of (Chitnis *et al*; 2000) and could lead to antiretroviral resistance (Nsagha *et al;* 2012). Regarding to the risk factors of contracting HIV infection among our HIV patients so the relative risk, and its confidence interval was calculated, and their significant also was determined by X^2 and p value. There was a significant association between HIV infection and history of injury (RR=2.4, CI=1.2-4.9, X^2=7.1, Pv=0.007), and history of other family member suffering from HIV infection (RR=2.1, CI=1.2-3.7, X^2=5.6, Pv=0.01) and this consistent with findings by (Open Society Institute; 2006), that prior factors were significant risk factors for HIV infections in Middle East. The significant association of injury and contracting HIV virus in our study reflect poor measures in screening blood donors for HIV with inadequately materials and instruments for screening blood donors for virus infections in most hospitals in Yemen particularly in district hospitals and branches in the other governorates such as, Aden, Taiz, Mokalla and Hodedah. Also the association of injury and contracting HIV virus reflect poor hygienic practices with inadequately sterilized medical instruments and tools that used for our patients. The study shows that direct association was found between HIV infections and other family member suffering from HIV. This suggests that risk of HIV infection increased among family who had a member suffering from HIV due to long period of contact (Palella *et al*;1998). Since the bad wave of Arab uprising in Yemen which is offering environment for more civil war which lead to collapse of Health system with a poor transfusion of blood bags, poverty, and female sex workers could lead to increase in the incidence rate of HIV. The control and prevention of HIV/AIDS should not only be for the government alone, but also for individuals of the community, corporate organizations, companies, Non-Governmental Organizations (NGO'S,) etc. People should show care and love to persons living with HIV/AIDS therefore Stigma and discrimination should be discouraged at all levels, we should strongly opined.

CONCLUSION

HIV patients are over 20 years old, the prevalence of HIV-1 is much higher than HIV-2 and no pure HIV-2 infection was occurred and the highly significant effect were related to family member suffering from HIV infection and history of injury with contracting HIV, but no effect of blood transfusion, history of surgical operation, dental procedure, and history of traveling abroad and contracting HIV type 2. Therefore, prevention programs aimed to reduce HIV infection among society particularly man and women are the utmost requirements. Majority of the HIV positive subjects were HIV-1/HIV-2. More investigated including all governorates to study the HIV-1/HIV-2 co-infection; antiretroviral resistance.

CONFLICT OF INTEREST

This work has no conflict of interest to declare.

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