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Study of Patterns and Markers of Human Immune Deficiency Virus -1 (HIV-1) Progression and Unemployment Rate among Patients from Alexandria, Egypt

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Abstract

Middle East and North Africa (MENA) new HIV cases show the highest increase among all regions in the world. Even though Egypt has a low prevalence among the general population (< 0.02%), a national HIV epidemic occurs in certain population risk groups. The current study was conducted to asses clinical and immunological disease progression; following up viral load (VL) and detecting delta-32 CCR5 genotype polymorphism in selected cases, determining unemployment rate and identify predictors of employment for HIV-cases. A cross sectional design was adopted. HIV infected cases attending Alexandria Fever Hospital (AFH) for one year. Interview questionnaire and four CD+4 counts were done for all patients, HIV VL and delta-32 CCR5 polymorphism were done for selected cases. Sexual transmission and drug abuse are the most important risk factors. Infectious comorbidity increases the rate of HIV progression. CD4+ count at the end of the study; CD+4 (4), count was significantly higher than all other CD4+ readings among the whole cohort and among the treated group. Also, VL at the end of the study; VL(2), was significantly higher than VL(1) among the untreated group. Unemployment rate was 40%. Male gender and obtaining vocational training were significant predictors of employment. It can be concluded that having a family member living with HIV and drug abusers are high risk groups for HIV acquisition. Factors responsible for progression of HIV should be further investigated. Antiretroviral therapy is very effective in checking HIV replication rate, delaying the progression of HIV, reconstituting the immune response and should be available for all cases detected.

Key words: Delta-32 CCR5 sequencing, HIV progression patterns, HIV-RNA viral load, HIV virus, unemployment rate among HIV cases

Introduction

Globally people living with HIV (PLHIV) numbered around 36.7 million at the end of 2015 according to the latest United Nations Acquired Immune Deficiency Syndrome (UNAIDS) 2016 data covering 160 countries (Global AIDS Update 2016 | UNAIDS, 2016). The burden of the epidemic continues to vary considerably between countries and regions. New HIV infections in the Middle East and North Africa (MENA) region have increased by 31% since 2001, which is the highest increase among all regions in the world (Gokengin *et al.*, 2016).

In Egypt, the estimated number of PLHIV is around 11,000 individuals (7,000–19,000) with overall low prevalence among the general population (< 0.02%). Greater Cairo and Alexandria; which have about one third of the population of the country, account for almost two thirds of all HIV infected patients detected. Egypt has a national epidemic concentrated in two key populations; people who inject drugs (PWID) and men who have sex with men (MSM) (Egypt National AIDS program NAP, 2015). Egyptian National guidelines on clinical care and antiretroviral therapy (ART) states that Care begins with CD4 cell count testing and those with CD4 count of 500 or lower are eligible for ART (National Guideline on Clinical Care and Antiretroviral Drugs for Treating and Preventing HIV Infection, Egypt, 2014).

HIV has a variable rate (spectrum) of progression in infected people. This variation in disease development is one parameter that led to the classification of patients as rapid progressors (RPs), slow/intermediate progressors, controllers; elite controllers (EC), viraemic controllers

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(VC) and long-term nonprogressors (LTNPs). The classification is also based on CD4 count, HIV viral load (VL), and ART. Different studies suggested different definitions to these subgroups (Gurdasani *et al.*, 2014).

Understanding mechanisms for significantly delaying disease progression remains a worthwhile research goal. Nonprogression probably results from multiple virologic, immunologic, and genetic factors. In terms of host genetics, some LTNPs display polymorphisms in chemokine receptors (CCR5 and CXCR4) and chemokines (*e.g.* MIP-1α and RANTES). Nearly 11% of Caucasians and 2% of Blacks are homozygous for the CCR5-delta32 mutation (Poropatich and Sullivan, 2011).

Employment status is a major predictor of health status and living conditions. However, unemployment rate among HIV – cases ranges from 45% to 65%. It is still higher compared with the general population, in many countries including highincome ones (Legarth *et al.*, 2014). Women, younger age, poor educational level, progressed HIV infections, or Hepatitis C virus (HCV) co-infection are risk factors for unemployment among HIV-cases (Gros *et al.*, 2016).

Our study aimed at (1) assessing clinical and immunological disease progression; (2) evaluating and following up the VL of selected cases, (3) detecting delta-32 CCR5 genotype polymorphism in selected cases, (4) determining unemployment rate and identify predictors of employment for HIV-cases.

Experimental

Material and Methods

A cross sectional design was adopted. All HIV infected cases attending Alexandria Fever Hospital (AFH) during field work period of the study from January to December 2015, who agreed to participate in the study were included (n = 150). Patients were excluded if they were younger than 18 years or refused to participate. They were diagnosed earlier by AFH protocol for HIV diagnosis (National Guideline on Clinical Care and Antiretroviral Drugs for Treating and Preventing HIV Infection, Egypt, 2014).

Study tools. An interview questionnaire. A predesigned questionnaire was used to obtain data from all HIV infected patients (n = 150) concerning a) sociodemography, b) risk factors, c) health status, d) history of the disease and e) treatment regimens of the participants.

Laboratory investigations

Blood samples were collected and analyzed at the AFH and Alexandria Faculty of Medicine (AFM) Central Laboratories.

The immunological status of the participants (CD4 count). CD4 + T-cell count (cells/mm^3) was taken as an immunological marker in the course of disease progression. Five CD4 counts were performed for all patients (n = 150): CD4+ (0); earliest CD4+ T-cell count (on diagnosis of HIV), CD4+ (1); CD4+ measured at the beginning of the study, CD4+(2); CD4+ measured after 3 months of the study, CD4+(3); CD4+ measured after 6 months of the study, CD4+(4); CD4+ measured after 9 months of the study.

Whole blood (200 µl) was collected in EDTA vacutainer tubes (Becton-Dickinson Mt View, California,). The PartecCyFlow Counter® (Partec GmbH, Munster, Germany) for CD4+ T-cell enumeration was used for performing the CD4 count. The Partec CD4% Reagent kit consists of MEM-24; a monoclonal antibody, which recognizes human CD4 antigen (Manasa *et al.*, 2007).

HIV viral load. VL was done twice to selected cases (n = 20). First VL (VL1) was done at the beginning of the study and the second VL (VL2) was done after one year. In our study we were more puzzled by PLHIV not showing any sign or symptoms for variable duration of time despite of not receiving treatment. So, we choose 16 out of the 29 untreated cases in our cohort to be followed up according to their VL in conjunction with their CD4 count. The other 13 untreated cases were excluded for the following reasons, presence of co-morbid infective diseases (HBV/HCV), noncompliance, and unwillingness to participate. In scope of covering the whole spectrum of different patterns of progression, 4 treated interesting cases were also chosen. A case with CD4 rapid decline after HIV diagnosis that dramatically improved on ART. The parents; diagnosed 17 years ago, of a family with four children, all are PLHIV. Lastly a case diagnosed and untreated for 5 years ago then underwent treatment when its CD4 started to decline.

Plasma were separated from the blood samples within 4 hours of collection and stored in a −20°C freezer. RNA extraction was performed using Qiagen QIAamp viral RNA mini spin protocol according to manufacturer instructions.

The VL for each of these samples was determined using a US FDA-approved kit (Artus HIV-1 RG RT-PCR kit; Qiagen, Germany).

As described by Luft, 2011; primers were diluted to a stock concentration of 200 mM and stored at −20°C. A concentration of 2 mM for SYBR Green-based reactions was done. A final primer concentration of 10 picomoles/ul was able to provide high-quality and consistent results.

Gene specific primers for the most conserved region of HIV-1 gag gene were chosen (Genbank)
Forward: 5'-ACATCAAGCAGCCATGCAAAT-3',
Reverse: 5'-TACTAGTAGTTCCTGCTATGTC-3'.
Biosystems StepOne™ Real Time PCR system was used

with the following conditions: one cycle of reverse transcription at 45°C for 30 min, followed by one cycle of polymerase activation at 95°C for 5 min then 40 cycles of PCR amplification. Each cycle consisted of denaturation at 95°C for 15 s, annealing at 55°C for 15 s, and extension at 72°C for 30 s.

CCR5 genotyping. Eight cases that fulfilled the criteria of being LTNPs (according to CD4 count, VL, duration since diagnosis and never on ART) were chosen for genotyping for the CCR5-delta32-allele.

After the genomic DNA was extracted using QIAGEN-Blood-Midi-Kit (Qiagen, Germany), screening of the cases for the CCR5-delta32-allele was performed with a genomic PCR using primers flanking the site of the deletion (forward: 5'-CTCCCAGGAATCATCTTTAC-3', reverse: 5'-TCATTTCGACACCGAAGCAG-3'). The PCRs were performed in 50-µl volumes, each comprising 2× mixture containing 1.5 mM $MgCl_2$, 10 mM dNTPS, 2.5 U Taq DNA polymerase, 50 ng genomic DNA, and 20 µmol of each primer (Gero, 2011).

The amplification conditions were as follows: denaturation at 94°C for 1 min, followed by 35 cycles of PCR amplification. Each cycle consisted of denaturation at 94°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 30 s. Following the 35 cycles of PCR there was an extension for 10 min at 72°C.

Results were confirmed by direct sequencing using the BigDye® - Terminator - 1.1. - Cycle-Sequencing - Kit (Applied Biosystems, Germany).

Statistical analysis of the data. The collected data were coded and typed onto computer files. All analyses were performed using SPSS/Pc + software program version 20.0 (Kirkpatrick and Feeny, 2013). The level of significance selected for results was 5% ($\alpha = 0.05$). Analytic statistics were carried out using Chi-Square test (X^2), Fisher's exact test and Student t-test were used to reveal association between characteristics of HIV-infected patients and HIV progression.

Wilcoxon Signed Ranks test, Paired t-test were done to compare mean values of HIV immunological and virological markers reported at different points of time. Additionally, unemployment rate was calculated. Univariate analysis was conducted where basic sociodemographic and health status characteristics were examined for association with respect to employment status, using Chi-Square test (X^2), Fisher's exact test, and Monte Carlo test for categorical variables, and Student t-test for quantitative variables. All variables that were significantly associated with employment status in univariate analysis, were included in multivariate analysis using logistic regression model to investigate predictors of employment among the studied HIV-cases.

Ethical clearance. The protocol of the current study was approved by AFH, MOH and the Medical Research Ethics Committee at AFM. Objectives of the study, expected benefits, types of information to be obtained, procedures, and publication were explained to each participant and an informed written consent was obtained before participation in the study. Moreover, confidentiality of data was insured.

Results

Characteristics of the studied HIV-infected patients with respect to disease progression (n = 150). Two thirds of our study HIV infected patients were males (n = 100, 66.7%). Their age ranged from 18–62 years with mean age of (33.93 ± 9.25) years. The majority of our patients have urban residence (n = 142, 94.7%) with 46 of them (30.7%) clustered in Amryia district. As regarding HIV acquisition risk factors, family member living with HIV was present in 55.3% of cases (n = 83), of which 67.46% (n = 46) have a spouse having HIV. Addiction was found in about 29.3% (n = 44) while 24% (n = 36) were intravenous (IV) abusers.

Regarding HIV progression, based on CD4 count, duration since diagnosis and ART, RP accounted for 56% of the studied group followed by progressors, controllers and LTNP accounting for 24.7%, 12.7% and 6.7% respectively. Among the studied group 24.6% (n = 37) had infectious comorbidity (Hepatitis B and HCV). In the treated group (80%, n = 121), Truvada + Efavirnez was the treatment regimen in 71.1% of cases and Efavirnez + Zidavudine + Lamivudine in 28.9% in cases with comorbidity.

Among different parameters, the presence of comorbidity was significantly associated with disease progression ($X^2 = 6.61$; $p = 0.01$) (Table I).

Immunological makers; CD4 + T-cell count (cells/mm³) among the studied HIV-infected patients. CD4 + (4) was significantly higher than the other four readings [(CD4 + (0), CD4 + (1) CD4 + (2), CD4 + (3)] among all HIV infected patients; n = 150, and also among the treated group; n = 121, ($p = 0.00$ and $p = 0.00$ respectively) (Table II).

Viral Load – VL (IU/ml) among the studied HIV-infected patients. VL was taken as a virological marker in the course of disease progression. Twenty cases that were chosen, showing different patterns of progression; one was RP, three intermediate were progressors, eight were controllers, 8 were LTNPs. Two LTNPs cases had VL BDL (< 50 IU/ml). VL(2) was significantly higher than VL(1) when assessing patients with detectable VL; n = 18, and also when assessing untreated patients with detectable VL; n = 14, ($p = 0.00$ and $p = 0.00$ respectively) (Table III).

CCR5 genotyping. PCR fragment of 200 base pairs (bp) were detected for CCR5 gene. The eight LTNP selected cases were found to carry the wild type of CCR5.

Table I
Characteristics of the studied HIV-infected patients with respect to disease progression (n = 150)

Characteristics	Non-progressive[$] (n = 29)		Progressive[^] (n = 121)		Test of significance (*P* value)
	No.	(%)	No.	(%)	
Gender Male	19	(65.5%)	81	(66.9%)	$X^2 = 0.02$ (0.88)
Female	10	(34.5%)	40	(33.1%)	
Age (Years) (Mean ± SD)	33.97 ± 11.06		33.93 ± 8.81		t = 0.02 (0.98)
Residence Rural	2	(6.9%)	6	(5%)	[FE]P = 0.65
Urban	27	(93.1%)	115	(95%)	
History of blood transfusion	4	(13.8%)	13	(10.7%)	[FE]P = 0.74
Family member living with HIV	14	(48.3%)	69	(57%)	$X^2 = 0.72$ (0.39)
IV drug abuser	6	(20.7%)	30	(24.8%)	$X^2 = 0.21$ (0.64)
Employment status Unemployed	12	(41.4%)	48	(39.7%)	$X^2 = 0.02$ (0.86)
Employed	17	(58.6%)	73	(60.3%)	
Co-morbidity None	26	(89.7%)	79	(65.3%)	$X^2 = 6.61$ (0.01)*
Infectious[<] and/or non-infectious[#]	3	(10.3%)	42	(34.7%)	

Abbreviations: HIV – human immune deficiency virus; IV – intravenous; [#] – diabetes Mellitus, hypertension;
[<] – hepatitis C virus, hepatitis B virus; [$] – includes long-term non-progressive and controller;
[^] – includes progressive and rapidly progressive; X^2: Chi square test; FE: Fisher's Exact test; t: student t test;
SD: standard deviation; * – significant at $p \leq 0.05$ (2-tailed)

Table II
Immunological makers; CD4 + T-cell count (cells/mm^3) among the studied HIV-infected patients

CD4 + T-cell count among all HIV-infected patients (n = 150)			
	Min-Max	Mean ± SD	Test of significance (p-value)
CD4+ (0)	28 – 1555	438.35 ± 259.15	Z" = –8.66 (0.00)**
CD4+ (1)	28 – 1555	474.14 ± 294.32	Z[$] = –8.11 (0.00)**
CD4+ (2)	48 – 1560	484.50 ± 283.11	Z[^] = –8.48 (0.00)**
CD4+ (3)	67 – 1500	495.72 ± 279.55	Z[@] = –7.95 (0.00)**
CD4+ (4)	74 – 1523	506.45 ± 279.21	
CD4 + T-cell count among HIV-infected patients who received treatment (n = 121)			
	Min-Max	Mean ± SD	Test of significance (p-value)
CD4+ (0)	28 – 732	360.60 ± 166.30	t" = –7.61 (0.00)**
CD4+ (1)	28 – 815	367.09 ± 170.26	t[$] = –10.06 (0.00)**
CD4+ (2)	48 – 820	382.83 ± 166.35	t[^] = –7.91 (0.00)**
CD4+ (3)	67 – 859	397.23 ± 168.15	t[@] = –5.46 (0.00)**
CD4+ (4)	74 – 836	407.27 ± 165.62	

Table II
Continued

CD4 + T-cell count among HIV-infected patients who did not receive treatment (n = 29)			
	Min-Max	Mean ± SD	Test of significance (p-value)
CD4+ (0)	369 – 1555	762.72 ± 322.13	Z" = –1.82 (0.06)
CD4+ (1)	369 – 1555	920.79 ± 283.90	t[$] = 0.02 (0.97)
CD4+ (2)	412 – 1560	908.72 ± 277.28	t[^] = –0.58 (0.56)
CD4+ (3)	481 – 1500	906.65 ± 279.10	t[@] = –1.12 (0.27)
CD4+ (4)	521 – 1523	920.27 ± 278.25	

Abbreviations:
HIV – human immune deficiency virus;
Z – Z value of Wilcoxon Signed Ranks test; t: paired t-test;
D: standard deviation; ** – significant at $p \leq 0.01$ (2-tailed);
" – CD4+ (0) – CD4+ (4); [$] – CD4 + (1) – CD4 + (4);
[^] – CD4 + (2) – CD4 + (4); [@] – CD4 + (3) – CD4 + (4);
CD4 + (0) – earliest CD4 + count;
CD4 + (1) – CD4 + measured at the beginning of the study;
CD4 + (2) – CD4 + measured after 3 months of the study;
CD4 + (3) – CD4 + measured after 6 months of the study;
CD4 + (4) – CD4 + measured after 9 months of the study

Characteristics of the studied HIV-infected patients with respect to patients' employment status (n = 150). Unemployment rate among HIV-infected patients was 40%. Most of employed HIV-infected patients were men (85.6%), while most of unemployed group were women (61.7%) ($X^2 = 36.12$; p = 0.00). Additionally, 41.1% and 6.7% of employed HIV-infected patients obtained vocational training and secondary school education respectively, compared with unemployed group (11.7% and 5% respectively) ([Monte Carlo] p = = 0.00). Significantly higher percentage of unemployed HIV-infected patients had family member living with

Table III
Viral load (IU/ml) among the studied HIV-infected patients

	VL among all HIV-infected patients (n = 20)			
	No.	%	Mean ± SD	Test of significance (p value)
VL (1) < detection level[d]	2	10	–	$Z^{\&} = -2.84$ (0.00)**
> detection level[d]	18	90	6990 ± 8593.65	
VL (2) < detection level[d]	2	10	–	
> detection level[d]	18	90	8010.22 ± 10193.1	
	VL among HIV-infected patients who received treatment (n = 4)			
	Min-Max		Mean ± SD	Test of significance (p value)
VL (1)	15400 – 27000		19570 ± 5366.40	
VL (2)	12000 – 34000		21450 ± 9187.49	
	VL among HIV-infected patients who did not receive treatment (n = 16)			
	No.	%	Mean ± SD	Test of significance (p value)
VL (1) < detection level[d]	2	12.5	–	$Z^{L} = -3.18$ (0.00)**
> detection level[d]	14	87.5	3396.14 ± 5227.32	
VL (2) < detection level[d]	2	12.5	–	
> detection level[d]	14	87.5	4170.29 ± 6703.13	

Abbreviations:
HIV – human immune deficiency virus; VL – viral load; Z – Z value of Wilcoxon Signed Ranks test;
** – significant at $p \leq 0.01$; VL (1) – VL measured at the beginning of the study;
VL (2) – VL measured after one year of the study; [d] – detection level was 50 IU/ml;
[&] – VL (1) – VL (2) for HIV patients whose viral load was above detection level (n = 18);
[L] – VL (1) – VL (2) for HIV patients whose viral load was above detection level (n = 14)

HIV (68.3%) compared with employed group (46.7%) ($X^2 = 6.83$; $p = 0.00$). Moreover, 34.4% of employed HIV-infected patients were PWID compared with 8.3% of unemployed group ($X^2 = 13.45$; $p = 0.00$). According to HIV progression, 17.8%, 63.3%, 4.4%, and 14.4% of employed HIV-infected patients had progressive, RP, LTNP, and controllers respectively, compared with unemployed patients (35%, 45%, 10% and 10%, respectively), ($^{\text{Monte Carlo}}$ $p = 0.03$) (Table IV).

Predictors of employment in the studied HIV-infected patients (n = 150) (Table V). Characteristics that were significantly associated with respect to employment status in univariate analysis were included in multivariate analysis using logistic regression model. The overall model was significant (model $X^2 = 57.11$, $p = 0.00$); significant predictors of employment were male gender (odds ratio [OR]: 7.29; 95% confidence interval [CI]: 2.72–19.52), and obtaining vocational training (OR: 6.04; 95%CI: 1.81–20.16). According to the results, HIV-infected man was 7.3 times more likely to be employed compared with HIV-infected woman. Moreover, HIV-infected patient who obtained vocational training was 6 times more likely to be employed than an illiterate HIV-infected patient. Although, in univariate analysis, having a family member living with HIV, PWID and HIV progression was significantly associated with employment; yet, these associations were no longer significant after adjustment for other covariates in multivariate analysis (Table V).

Discussion

The world has committed to ending the AIDS epidemic by 2030 as declared by UNAIDS 2016. The 90-90-90 target provides that by 2020: (a) 90% of all PLHIV will know their HIV status; (b) 90% of all people with diagnosed HIV infection will receive sustained antiretroviral therapy; and (c) 90% of people receiving antiretroviral therapy will achieve viral suppression (Global gains made towards the 90-90-90 targets UNAIDS, 2016)

In the present study males accounted for two thirds of cases. Similarly, 83% of HIV infected patients

Table IV
Characteristics of the studied HIV-infected patients with respect to patients' employment status (n = 150)

Characteristics	Employed HIV patients (n = 90)		Unemployed HIV patients (n = 60)		Test of significance (p value)
	No	(%)	No	(%)	
Gender					
Male	77	(85.6)	23	(38.3)	$X^2 = 36.12\ (0.00)^{**}$
Female	13	(14.4)	37	(61.7)	
Age (Years) (Mean ± SD)	35 ± 9.18		32.32 ± 9.18		t = 1.76 (0.08)
Marital status					
Single	41	(45.6)	19	(31.7)	$^{MC}p = 0.12$
Married	31	(34.4)	27	(45)	
Divorced	15	(16.7)	8	(13.3)	
Widowed	3	(3.3)	6	(10)	
Residence					
Rural	7	(7.8)	1	(1.7)	$^{FE}p = 0.14$
Urban	83	(92.2)	59	(98.3)	
Education					
Illiterate	19	(21.1)	19	(31.7)	$^{MC}p = 0.00^{**}$
Read & write	12	(13.3)	10	(16.7)	
Primary	6	(6.7)	10	(16.7)	
Secondary	10	(11.1)	11	(18.3)	
University	6	(6.7)	3	(5)	
Vocational training	37	(41.1)	7	(11.7)	
Family member living with HIV	42	(46.7)	41	(68.3)	$X^2 = 6.83\ (0.00)^{**}$
IV drug abuse	31	(34.4)	5	(8.3)	$X^2 = 13.45\ (0.00)^{**}$
Duration since HIV diagnosis					
< 5 years	33	(36.7)	25	(41.7)	$X^2 = -0.41\ (0.81)$
5 – 15 years	48	(53.3)	30	(50)	
> 15 years	9	(10)	5	(8.3)	
Co-morbidity$^{<}$	31	(34.4)	14	(23.3)	$X^2 = 2.11\ (0.14)$
Symptomatic HIV	71	(78.9)	50	(83.3)	$X^2 = 0.45\ (0.50)$
Type of progression					
Progressive (P)	16	(17.8)	21	(35)	$^{MC}p = 0.03^{*}$
Rapidly progressive (RP)	57	(63.3)	27	(45)	
Long-term non-progressive	4	(4.4)	6	(10)	
Controller	13	(14.4)	6	(10)	
CD4+ T cells (cells/mm^3)^					
< 100	7	(7.8)	8	(13.3)	$^{MC}p = 0.13$
100–199	3	(3.3)	6	(10)	
200–349	21	(23.3)	8	(13.3)	
≥ 350	59	(65.6)	38	(63.3)	
Viral load (IU/ml)$^{\$}$	(n = 11)		(n = 9)		$^{FE}p = 1$
< 10000	8	(72.7)	6	(66.7)	
≥ 10000	3	(27.3)	3	(33.3)	
Receiving cART	73	(81.1)	48	(80)	$X^2 = 0.02\ (0.86)$

Abbreviations:
HIV – human immune deficiency virus; st. – status; $^{<}$ – diabetes mellitus, hypertension, hepatitis C virus, hepatitis B virus; cART-combined antiretroviral therapy; ^ – baseline CD4 T-helper counts; X^2 – Chi square test; FE – Fisher's Exact test; MC – Monte Carlo test; t – student t-test; SD – standard deviation; * – significant at $p \leq 0.05$ (2-tailed); ** – significant at $p \leq 0.01$ (2-tailed)

Table V
Predictors of employment in the studied HIV-infected patients (n = 150)[a]

Characteristics	Multivariate analysis (logistic regression)			
	β	OR[#]	95%CI	p value
Gender				
Female^	–	–	–	–
Male	1.98	7.29	2.72–19.52	0.00**
Education				
Illiterate^	–	–	–	–
Read and write	0.60	1.82	0.50–6.58	0.35
Primary	–0.19	0.82	0.20–3.33	0.78
Secondary	0.07	1.07	0.30–3.85	0.91
University	0.94	2.57	0.42–15.57	0.30
Vocational training	1.79	6.04	1.81–20.16	0.00**
Having family member living with HIV				
Yes	0.16	1.17	0.45–3.07	0.74
No^	–	–	–	–
IV drug abuse				
Yes	0.93	2.54	0.78–8.28	0.12
No^	–	–	–	–
Disease progression				
Progressive	–1.23	0.29	0.06–1.22	0.09
Rapidly progressive	–0.515	0.59	0.16–2.22	0.44
LTNP	–1.09	0.33	0.04–2.39	0.27
Controller^	–	–	–	–
Model X^2 = 57.11 (0.00)**				

Abbreviations:
HIV – human immune deficiency virus; LTNP – long term non-progressive; IV – intravenous;
[a] – Multivariate odds ratios using logistic regression; ^ – Reference;
[#] – Odds ratios adjusted for all variables listed in the table; X^2 – Chi square; ** – significant at $p \leq 0.001$

reported were males with same age range in an Egyptian survey (Jackobsen, 2014).

The majority of our patients had urban residence (94.7%) and Amrya district showed the highest clustering (30.7%). Amrya district was also found to be the second highest district of HCV clustering in a PhD study conducted at the AFM (Gamaleldeen, 2016).

Sexual transmission and drug abuse remain the major routes of HIV infection in MENA including Egypt (Gokengin *et al.*, 2016). Our results of HIV due to IV abuse is in harmony with the results of a national surveillance study showing that 28% of reported HIV infected patients in Egypt in 2010 occurred in PWID which is consistent with an epidemic that disproportionately affects MSM and/or PWID (NAP, HIV/AIDS biological and behavioral surveillance; 2010). Male PWID comes out to 93,314; 0.37% of the male population aged 18–59 (Jackobsen, 2014).

In the last few years, many countries in MENA have been affected by social and political unrest and conflict. However, the current HIV prevalence of 0.1% is still among the lowest rates globally (El Beih *et al.*, 2012). Iran has the highest numbers of PWID (185 000, 0.43%), followed by Pakistan (117 000) and Egypt (93 000) (Mumtaz *et al.*, 2014).

HCV is a common co-infection among PLHIV in Egypt. This is due to the high background of HCV prevalence in the general population (7%) (EHIS, 2015). As in most countries, Egypt is adopting nucleic acid testing in blood banks to provide safe blood transfusions (Egyptian national standards for blood transfusion, 2011).

The development of signs and symptoms of AIDS typically parallels laboratory testing for CD4 lymphocytes. CD4 lymphocyte count, HIV VL and follow up duration are key components used to define HIV phenotypes (Gurdasani *et al.*, 2014).

In our study group, RP accounted for 56% while average percent is around 10–15% (Gurdasani *et al.*, 2014). This could be attributed to that those patients have signs and symptoms so they are more keen to show up for treatment and follow up. Also, PLHIV is

still a stigma as described in the Egyptian society for population studies (Stigma experienced by people living with HIV in Egypt, 2013).

In the current study, LTNP accounted for 6.7% which is similar to average estimates (5–10%) (Gurdasani *et al.*, 2014). EC proportion varies greatly between studies, it ranged from 0.15–7.70% according to the Concerted Action on SeroConversion on AIDS and Death in Europe (CASCADE) dataset as it did not necessarily reflect the length of follow-up required by the definition.

The best laboratory measure for determination of the long term progression of AIDS for therapeutic purposes is the level of HIV RNA in peripheral blood. Despite ART, the reconstitution of the immune system may be partial or incomplete, with considerable variability in the magnitude of the response (Gunthard *et al.*, 2016). CD4 count response is controversial, it mostly increases but still may remain below normal.

In our study, the significantly higher CD4+(4) count among the whole cohort and among the treated group as well as significantly higher VL (2) among the untreated group intensify the importance for ART in HIV patients.

CCR5 is a prominent cofactor for HIV-1 entry. 74 mutations including the intensively studied 32 base pair deletion (CCR5-delta32) were identified. We detected no deletion mutations among selected LTNP cases. Mutation occurs most frequently in the Caucasian population, while it cannot be found in the Asian, Middle East, African, and the American Indian population (Gero *et al.*, 2011).

In the present study, the unemployment rate among HIV-infected patients was 40%. The result coincides with unemployment rates of 45–65% reported by Dray-Spira *et al.* (2007) and 40% reported by Rabkin *et al.* (2004). However, it is much higher than unemployment rate; 12.1%, among HIV-infected patients reported in Gros *et al* (2016) which could be attributed to national difference; the overall unemployment rate in Rabkin *et al.* (2004) in Germany (4.6%) is lower than that in Egypt according to ILO, 2016 (12.7%). Yet, results of the current research are consistent with recent studies that indicates higher unemployment rates of HIV-infected individuals compared with the background population.

On studying predictors of employment for HIV-infected patients, HIV-infected men were more likely to be employed than HIV-infected women. Likewise, Dray Spira *et al.* (2007), reported lower rates of employment among HIV-infected women (46.9%) compared with HIV-infected men (65.1%), however, in the current study, employment rate among women was very much lower than Dray Spira *et al.*, 2007 (14.4% *vs* 46.9%). This could be partially due to the fact that women, even in absence of HIV disease, have lower opportunity regarding labor market participation especially in developing countries. In the general population in Egypt, unemployment among females is 25.7%, compared with 8.9% in males (CAPMAS, 2016).

Furthermore, in the present research, HIV-infected patients who obtained vocational training were more likely to be employed compared with illiterate HIV-infected patients. Similarly, previous studies showed higher probability of labor market participation in highly educated HIV-infected individuals as described by Elzi (2016) and that poor education is significantly associated with higher unemployment rate among HIV-infected patients (Gros *et al.*, 2016).

According to HIV/AIDS Strategic Frame 2012–2016; Egypt's goal is to stabilize the growth of the AIDS epidemic in the country, prevent new infections especially within the most at risk population and improve health outcomes for PLHIV. National positive response developments; most notably prevention programmes for the key populations of PWID and MSM, have been implemented. HIV testing is available for diagnostic purposes and both governmental and nongovernmental voluntary testing and counselling are being expanded. Treatment centers have been set up at fever hospitals. Stigma and discrimination are prohibited and various care and supportive measures are undertaken.

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